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Assessment of Thyroid Functions and Serum Autoantibodies as Diagnostic Tools of Nonneoplastic Thyroid Disease Patients in Shendi Locality - Sudan

A Thesis Submitted in Fulfillment for the Requirements of the PhD Degree in Clinical chemistry

By

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بسم الله الرحمن الرحيم

(اللّهُ لاَ إِلَـهَ إِلاَّ هُوَ الْحَيُّ الْقَيُّومُ لاَ تَأْخُذُهُ سِنَةٌ وَلاَ نَوْمٌ لَّهُ مَا فِي السَّمَاوَاتِ
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DECLARATION OF AUTHORSHIP

I hereby declare that this thesis has been composed entirely by myself with the assistant of the supervisors, and is a result of my own interpretations and investigations. When I have consulted and quoted from the published work of others, this is always clearly attributed. It has neither been accepted nor submitted for any other degree in this university or any other academic institution. The data collection, analysis and interpretation were the sole work of the author, except where acknowledged.

The writing of this thesis is the sole work of the author. All sources of information and help have been fully acknowledged.

Name: - Abd Elwahab Abdien Saeed Taha

Signature:-……...

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Dedication

This Project is dedicated to

the soul of My Father …….

Who gave me the meaning of the life

And to My Mother (Fatima Elkhair)

My Lovely wife and Daughters (Lodan and Hala)

My sisters and My brothers

My friends and My colleagues……..

The persons whom I love, respect and appreciate…..

To all who has ever taught me anything

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Abstract

Thyroid disorders are the second most common problems in society and there are environmental, immunological and genetic factors that lead to the development of thyroid disorders.

The aim of this study was to evaluate thyroid hormones and antibodies in the diagnosis of thyroid diseases and to compare the thyroid test between patients and healthy control group, compare the values of antibodies against the thyroid gland with diagnostic reference values and also compare the values of hormone measurements with the diagnostic values of antibodies, and to evaluate the prevalence of antibodies of thyroid hormones in patients with thyroid disorders in Shendi locality and also to compare thyroid hormone values with symptoms in patients.

Total of (183) clinical specimens were collected from patients with thyroid disorders identified by the internal medicine specialist from Al-Mek Nemir University Hospital and from referral clinics and outpatient clinics from 2013 to 2017. Blood samples were taken from patients after explaining the purpose of the study, and after the questionnaire was completed by the doctor, these samples were tested for thyroid hormones and antibodies using the latest methods and advanced devices (TOSOH and ELIZA).

Obtained results were analyzed using the statistical package for social sciences, SPSS program, to analyze the study data.

The study showed that (60.7%) had hypothyroidism, (39.3%) had hyperthyroidism, (91.9%) of hypothyroidism were female, and only (8.1%) were male, while (84.7%) of hyperthyroidism were female while male represented only (15.3%) of them.

The study also showed that (33.3%) of hypothyroidism patients had a family history, (64.9%) of them were first degree, (35.1%) had a family history of second degree and (66.7%) had no family history of the disease. (37.5%) of hyperthyroidism patients had a family history, (70.4%) of them with family history of first degree, (29.6%) had a family history of the second degree, and (62.5%) had no family history. We also found that about half of these patients were newly discovered. The age of these patients ranged from (50.4 ± 14.7 years) in patients with hypothyroidism and (43.6 ± 13.4 years) with hyperthyroidism.

There were statistically significant differences between the values of thyroid hormones and TSH when combined with hyperthyroidism, hypothyroidism and control sample (P.value 0.000).

The study also showed that (58.5%) of patients were positive for TPOAb antibodies, (64.9%) of patients with hypothyroidism were positive of this antibody and (48.6%) of hyperthyroidism patients. For TgAb antibody, (39.9%) are positive, (69.9%) had hypothyroidism and (30.1%) had hyperthyroidism.

The study also showed that there was a statistically significant positive relationship between the presence of TPOAb and the values of fT3 and the presence of some symptoms such as fever, fatigue, increased appetite and tremor, while there was relationship with the sweating.

TgAb: there is an inverse relationship between them and some symptoms such as tremor, weight loss and sweating, while there is a direct relationship with loss of appetite and diet and some eye symptoms.

There was also a positive correlation between the values of thyroid hormones and some clinical symptoms such as diarrhea and tachycardia.

The study also showed the majority of clinical symptoms in patients with hypothyroidism associated with the level of hormones of the gland more than antibodies, there was also some specific hyperthyroidism feature appeared in patients with hypothyroidism and the study showed that it is statistically significant with the presence of thyroid antibodies.

Key words: thyroid gland, thyroid hormones, thyroid dysfunction, hypothyroidism, hyperthyroidism, thyroid autoantibodies, TSH, TT4, fT4, TT3, fT3, TPOAb, TgAb.

ملخص البحث

تمثل اضطرابات الغدة الدرقية من اكثر المشاكل في المجتمع وهنالك عوامل بيئية ومناعية وجينية تؤدي لتطور اضطرابات الغدة الدرقية

تهدف هذه الدراسة الي تقييم هرمونات الغدة الدرقية والاجسام المضادة في تشخيص امراض الغدة الدرقية و مقارنة اختبارت الغدة الدرقية بين المرضى والاصحاء، مقارنة قيم الاجسام المضادة للغدة الدرقية مع القيم المرجعية التشخيصية وايضاً مقارنة قيم قياسات الهرمونات مع القيم التشخيضية للاجسام المضادة، وتقييم معدل انتشار الاجسام المضادة لهرمونات الغدة الرقية لدي مرضى اضطرابات الغدة الدرقية في محلية شندي وايضاً مقارنة قيم هرمونات الغدة الدرقية مع الاعراض التي تظهر عند المرضى.

جمع عدد 183 عينة من العينات السريرية من المرضى الذين يعانون من اضطرابات الغدة الدرقية والتي تم تحديدهم بواسطة اختصاصي الطب الباطن من مستشفى الملك نمر الجامعي و من عيادات المحولة والعيادات الخارجية في الفترة من 2013 – 2017 وتم أخذ عينات دم من المرضى بعد شرح الغرض من البحث وابداء موافقتهم وبعد ملء الاستمارة بواسطة الطبيب وتم فحص هذه العينات لهرمونات الغدة الدرقية والاجسام المضادة وذلك بواسطة احدث الطرق والجهزة المتطورة (جهاز ال **TOSOH** و جهاز **ELIZA**)

حللت نتائج الفحوصات احصائياً باستخدام الحزمة الإحصائية للعلوم الاجتماعية الذي يعرف ببرنامج (SPSS) لتحليل بيانات الدراسة.

أظهرت الدراسة ان 60.7% كانو يعانون من نقص نشاط الغدة الدرقية و 39.3% يعانون من فرط نشاط الغدة الدرقية، و91.9% من مرضى نقص نشاط الغدة الدرقية كانو من النساء وفقط 8.1% كانو من الرجال، بينما 84.7% من مرضى فرط نشاط الغدة الدرقيو كانو من النساء بينما الرجال كانو يمثلون فقط 15.3% منهم. وايضا الدراسة اوضحت ان 33.3% من مرضى نفص نشاط الغدو الدرقية كان لديهم تاريخ عائلي و 64.9% منهم من الدرجة الاولي و 35.1% لديهم تاريخ مرضي عائلي من الدرجة الثانية و 66.7% ليس لديهم اي تاريخ عائلي للمرض، اما بالنسبة لمرضى فرط نشاط الغدة الدرقية فان 37.5% كان لديهم تارخ عائلي 70.4% منهم لديهم تاريخ عائلي من الدرجة

الاولى و 29.6% لديهم تاريخ عائلي من الدرجة التانية بينما 62.5%ليس لديهم اي تاريخ عائلي. وايضا وجدنا ان حوالي نصف هؤلاء المرضي تم اكتشافهم لاول مرة. اعمار هؤلاء المرضى تتراوح بين 14.7 ±50.4 سنة عند مرضي نقص نشاط الغدة الدرقية و 13.4±43.6 سنة.

هنالك فروقات ذات دلالة احصائية بين قيم هرمونات الغدة الدرقية والهرمون المحفز المفرز من الغدة النخامية عند مقرنتها لدي مرضى فرط نشاط الغدة ومرضى نقض النشاط والعينة الضابطة.

ايضاً اوضحت الدراسة ان 58.5% من المرضى كانو موجبي الاجسام المضادة TPOAb، و64.9% من مرضى نقص نشاط الغدة الدرقية كانو موجبي هذا الجسم المضاد و 48.6% من مرضى فرط النشاط، اما بالنسبة للجسم المضاد من النوع TgAb فان 39.9% من المرضي كانو موجبي الجسم المضاد، 69.9% منهم كانو يعانون من نقص نشاط الغدة و 30.1% منهم يعانون من فرط نشاط الغدة الدرقية.

اظهرت الدراسة ايضاً عند مرضي فرط نشاط الغدة الدرقية: ان هنالك علاقة ذات دلالة احصائية بين وجود TPOAb و قيم fT3 ووجود بعض الاعراض مثل الحمى، الاعياء وازدياد الشهية والارتعاش بينما هنالك علاقة عكيسة مع التعرق.

اما بالنسبة لل **TgAb:** فان هنالك علاقة عكسية بينها وبين بعض الاعراض مثل الرعشة و فقدان الوزن والتعرق، بينما هنالك علاقة طردية مع فقدان الشهية و الحمي وبعض اعراض العيون.

ايضاً هنالك علاقة طردية بين قيم هرمونات الغدة الدرقية مع بعض الاعراض السريرية مثل الاسهال وزيادة ضربات القلب

اوضحت الدراسة ايضاً غالبية الأعراض السريرية عند مرضى نقص نشاط الغدة الدرقية لها علاقة مع مستوي هرمونات الغدة اكثر من الاجسام المضادة، ايضاً هنالك ظهرت اعراض خاصة بمرضى فرط النشاط للغدة الدرقية عند هؤلاء المرضى وأظهرت الدراسة انها ذات دلالة احصائية مع وجود الاجسام المضادة لهرمونات الغدة الدرقية.

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List of Abbreviations

Abbreviation	Full Name
AH	Autoimmune hypothyroidism
ANS	8 – anilino – 1 – naphthalene sulfonic acid
AIA	Automated Immuno assay
AIH	Autoimmune hepatitis
AIT	Autoimmune Thyroid disease
APCs	Antigen presenting cells
ATD	Autoimmune thyroid disease
CAPZB	F-actin-capping protein subunit beta
CNS	Central nervous system
CPK	Creatine Phosphokinase
CT	Computed Tomography
CTLA4	Cytotoxic (T-lymphocyte antigen-4)
CTLAF-4	Cytotoxic T lymphocyte associated factor 4
DIO1	Iodothyronine deiodinase 1
DIT	Diiodothyronine
DIT	Diiodotyrosine
DM	Diabetes mellitus
DNA	Deoxyribonucleic acid
ELISA	Enzyme - linked immunosorbent assay
ERK1/2	Extracellular signal-Regulated Kinase ½
FCRLs	FC-receptor-like genes
FNA	Fine-Needle Aspiration
fT3	Free Triiodothyronine
fT4	Free Thyroxine
GD	Graves' disease
GWAS	Genome-Wide Association Studies
HADS	Hospital anxiety and depression scale
HapMap	Haplotype Mapping
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HLA	Human leukocyte antigen
HT	Hashimoto's thyroiditis

Abbreviation	Full Name
I^-	Iodide
I^0	Iodine
ICCIDD	International Council for Control of Iodine Deficiency Disorders
IDDs	Iodine Deficiency Disorders
IFIH1	Interferon-induced helicase-1
IRMA	Immunoradiometric assay
KDa	Kilo Dalton
LFT	Liver function test
LOD	Logarithm of odds
4MUP	4 – Methylumbelliferyl phosphate
MCT8	Monocarboxylate transporter 8
MHC	Major histocompatibility complex
MIT	Monoiodothyronine
MIT	Monoiodotyrosine
MMI	Methimazole
MRI	Magnetic Resonance Imaging
mRNA	Messenger Ribonucleic Acid
Na/I	Sodium -iodide
NHANES	National Health and Nutrition Examination Survey
NIS	Sodium/iodide (Na^+/I^-) symporter
OATP1C1	Organic-anion transporting polypeptide 1C1
OR	Odds ratio
PC	Papillary carcinoma
PDE8B	Phosphodiesterase 8B
PTH	Parathyroid hormone
PTPN22	Lymphoid tyrosine phosphatase-22
PTPN22	Protein tyrosine phosphatase-22
PTU	Propylthiouracil
RAIU	Radioactive Iodine Uptake
RIA	Radioimmunoassay
rT3	Reverse Triiodothyronine
RXR	retinoid X receptor
SNPs	Single Nucleotide Polymorphisms

Abbreviation	Full Name
SPSS	Statistical Package for the Social Sciences
T3	Triiodothyronine
T4	Thyroxine
TBA	Thyroid-Binding Albumin
TBG	Thyroid-Binding Globulin
TBPA	Thyroxine - Binding Prealbumin
TC	Thyroid cancer
Tg	Thyroglobulin
TgAb	Thyroglobulin antibody
TPO	Thyroidal Peroxidase
TPOAb	Thyroidal Peroxidase antibody
TSI	Thyroid stimulating immunity
TSHR-Ab	Thyroid Stimulating Hormone receptor antibodies
TRH	Thyrotropin Releasing Hormone
TRs	Thyroid hormone receptors
TSAb	Thyroid-stimulating antibodies
TSH	Thyroid Stimulating Hormone
U.K	United Kingdom
UI	Urinary iodine
UNICEF	United Nations International Children's Emergency Fund
USA	United States of America
USI	Universal salt iodization
WHO	World Health Organization

CHAPTER ONE

Introduction
Rationale
Objectives

1.1 Introduction

Once diabetes is excluded, thyroid diseases constitute the main bulk of endocrine problems that the practicing physician has to sort it out during the clinical practice. [1]

Thyroid disease usually occurs between the ages of (30 to 50 years). The prevalence of overt hypothyroidism is about (19 per 1000 women) and (1 per 1000 men). Overt hyperthyroidism occurs in about (20 per 1000) women and (2 per 1000 men). [2]

Iodine is an essential micronutrient required for normal thyroid function, growth and development. When iodine intake falls below recommended levels, the thyroid may no longer be able to synthesize sufficient amounts of thyroid hormone. [3]

Iodine deficiency disorders were a significant problem in the U.S until the 1920s, when the general use of iodized salt was initiated. [4]

Thyroid Hormone first isolated in 1914 by Kendall, [5] and first synthesized in 1925 by Harrington, [6] thyroxine T4 is a classic hormone that is used worldwide to treat millions of patients with thyroid disorders. During the past decades much progress has been made in the understanding of thyroid hormone TH physiology and a substantial part of TH biology have been elucidated. T4 is the main secretory product of the thyroid gland. In humans, it comprises (~80%) of the THs secreted, the remaining (~20%) being secreted as triiodothyronine T3. T4 has only limited affinity for the nuclear thyroid hormone receptors THRs as compared with T3, which is regarded the primary biologically active form. In order to become bioactive, T4 has to be converted to T3 by outer-ring deiodination. Furthermore, both T4 and T3 can be inactivated by inner-ring deiodination. These reactions are catalyzed

by the iodothyronine deiodinases type 1, 2 and 3 (D1, D2 and D3) that are expressed in a multitude of peripheral tissues, each deiodinase with its specific tissue distribution. Outer ring deiodination, i.e. the activating pathway, is catalyzed by D1 and D2. Inner ring deiodination of T4 and T3 to lower iodothyronines that have no affinity for the THRs, i.e. the inactivating pathway, is catalyzed by both D1 and D3. [7]

Measurement of TSH has become the principal test for the evaluation of thyroid function in most circumstances. [8] A TSH value within the reference interval excludes majority of cases of primary overt thyroid disease. If TSH is abnormal, confirm the diagnosis with fT4. Where risk factors exist, consider fT3 when fT4 is normal and thyrotoxicosis is suspected. [9] The TSH level may be borderline elevated in the presence of normal levels of fT4. [10]

Measurements of fT4 and fT3 have replaced measurements of TT4 and TT3 levels. Laboratories are permitted to substitute free hormone assays when total T3 or T4 have been ordered. Measurement of fT3 in patients with suspected hyperthyroidism is rarely indicated. This is reserved for situations where hyperthyroidism is suspected clinically and TSH is suppressed, but the fT4 is not elevated, measurement of fT3 is not indicated in hypothyroidism. [11]

Thyroid peroxidase TPO: are the key thyroid enzyme catalyzing both the iodination and coupling reaction for the synthesis of the thyroid hormone. It is membrane bound and found in the cytoplasm and in high concentration on the apical microvillar surface of thyrocytes. It is of mol wt between (100 to 150 KDa) and previously was known as thyroid microsomal antigen, [12] multiple T – and B – cell epitopes exist within the molecule, and the

antibody response to TPO is restricted at the level of the germ line heavy and light chain variable V region. [13]

Anti-TPO autoantibodies are found in over (90%) of patients with autoimmune hypothyroidism and Graves disease. Together with thyroglobulin Tg antibodies these are the predominant antibodies in AH. Anti- TPO antibodies are mainly of the IgG class 1 and IgG4 subclasses in excess. [14, 15]

Thyroglobulin Tg: Tg is a (660-KDa) glycoprotein composed of two identical subunits of (330 KDa) each. It is secreted by the thyroid follicular cells into the follicular lumen and stored as a colloid substance within the thyroid follicles. Each Tg molecule has around (100) tyrosine residues, a quarter of which are iodinated. These residues couple to for triiodothyronine T3 and thyroxine T4. The sequence of human Tg has been determined (80). When TSH stimulates the thyroid cells, Tg is endocytoses and hydrolyzed in lysosome releasing T3 and T4. The exact location of T- and B- cell epitopes within Tg is uncertain. [16]

Thyroglobulin autoantibodies are found in less than (60%) of patients with lymphocytic thyroiditis and (30%) of Graves' disease patients. They are polyclonal and mainly of IgG class with all four subclasses represented. TSH regulates the cell surface expressions of TPO and Tg altering the transcription of these two proteins, possibly at the gene promoter level. These effects are mimicked by autoantibodies (both blocking and stimulating) in sera of patients with Graves' disease. [17]

Iodine Intake: Mild iodine deficiency is associated with lower prevalence of Hashimoto's disease and hypothyroidism, while excessive intake is associated with a higher prevalence. [18] As an example, in China,

autoimmune thyroiditis was found in (0.3%) of those with mildly deficient iodine intake and (1.3%) of those with excessive iodine intake. [19]

1.2 Rationale

In the Sudan, the period from the early 1980s to mid 1990s witnessed substantial activity in connection with iodine deficiency in the form of epidemiological and etiological studies and assessments of the effects of different interventions. Thyroiditis is a group of inflammatory thyroid disorders. Patients with chronic lymphocytic thyroiditis (also referred to as Hashimoto's thyroiditis) present with hypothyroidism, goiter, or both. Measurement of thyroid function test and serum thyroid autoantibodies and thyroglobulin confirms the diagnosis, graves' disease is the most common cause of primary hyperthyroidism, most likely due to autoimmune due to TSI antibodies, it is in need to evaluate the presence of thyroid autoantibodies; and the prevalence of autoimmune thyroid diseases (hyper and hypothyroidism) and thyroid autoantibodies levels in Shendi locality.

1.3 Objectives

1.3.1 General objective

To evaluate thyroid function tests and thyroid autoimmune antibodies among patients with nonneoplastic thyroid disease in Shendi Locality

1.3.2 Specific objectives

1.3.2.1 To compare thyroid function test between patients and control.

1.3.2.2 To compare estimated values of thyroid autoimmune antibodies with expected values.

1.3.2.3 To compare thyroid function tests with diagnostic values thyroid autoimmune antibodies among patients.

1.3.2.4 To evaluate the frequencies of autoimmune thyroid diseases depending on the presence of thyroid auto antibodies and their distribution in Shendi locality.

1.3.2.5 To compare thyroid hormones level & clinical features and findings among patient

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CHAPTER TWO

Literature Review

2. Literature Review

2.1 Thyroid gland

Etymology

The English name thyroid gland [20] is derived from Latin glandula thyreoidea. [21] Glandula means gland in Latin, [22] and thyreoidea can be traced back to the Ancient Greek word θυρεοειδής, meaning shield - shaped. [23]

The English anatomist Thomas Wharton was the first to coin the Latin expression for the thyroid gland. [24] However, he introduced the incorrect spelling glandula thyroidaea, [25] as the adjective thyroidaea is a faulty rendering in Latin of Ancient Greek θυρεοειδής. [26] The Latin ending aea does not correspond to Ancient Greek ής and more importantly the e after thyr is missing, creating a resemblance between thyroidaea and Ancient Greek θυροειδής that actually means like a door instead of the intended shield-like. [23]

2.1.1 Thyroid anatomy and development:

The thyroid gland is positioned in the lower anterior neck and has a shape similar to a butterfly. It is divided into two lobes, one on either side of the trachea. [27] Lobus dexter right lobe and lobus sinister left lobe, connected via the isthmus. Each lobe is about (5cm long), (3cm wide) and (2cm thick). [28]

The organ is situated on the anterior side of the neck, lying against and around the larynx and trachea, reaching posteriorly the oesophagus and carotid sheath. It starts cranially at the oblique line on the thyroid cartilage (just below the laryngeal prominence, or 'Adam's Apple'), and extends inferiorly to approximately the fifth or sixth tracheal ring. It is difficult to demarcate the gland's upper and lower border with vertebral levels because it moves position in relation to these during swallowing. There is occasionally (28-55%) of

population, mean (44.3%). [28] A third lobe present called the pyramidal lobe of the thyroid gland. It is of conical shape and extends from the upper part of the isthmus, up across the thyroid cartilage to the hyoid bone. The pyramidal lobe is a remnant of the fetal thyroid stalk, or thyroglossal duct. It is occasionally quite detached, or may be divided into two or more parts. The pyramidal lobe is also known as Lalouette's pyramid. [29]

The thyroid gland is covered by a thin fibrous sheath, the capsula glandulae thyreoideae, composed of an internal and external layer. The external layer is anteriorly continuous with the pretracheal fascia and posteriorolaterally continuous with the carotid sheath. The gland is covered anteriorly with infrathyoid muscles and laterally with the sternocleidomastoid muscle also known as sternomastoid muscle. On the posterior side, the gland is fixed to the cricoid and tracheal cartilage and cricopharyngeus muscle by a thickening of the fascia to form the posterior suspensory ligament of thyroid gland also known as Berry's ligament. [30, 31] The thyroid glands firm attachment to the underlying trachea is the reason behind its movement with swallowing. In variable extent, the pyramidal lobe is present at the most anterior side of the lobe. In this region, the recurrent laryngeal nerve and the inferior thyroid artery pass next to or in the ligament and tubercle. Between the two layers of the capsule and on the posterior side of the lobes, there are on each side two parathyroid glands. [32]

The thyroid isthmus is variable in presence and size, can change shape and size, and can encompass the pyramidal lobe (lobus or processus pyramidalis). The thyroid is one of the larger endocrine glands, weighing (2-3grams) in neonates and (18-60 grams) in adults, and is increased in pregnancy.

In a healthy patient the gland is not visible yet can be palpated as a soft mass. Examination of the thyroid gland is carried out by locating the thyroid cartilage and passing the fingers up and down, examining for abnormal masses and overall thyroid size. Then, place one hand on each of the trachea and gently displace the thyroid tissue to the contralateral side of the neck for both sides while the other hand manually palpates the displaced gland tissue; having the patient flex the neck slightly to the side when being palpated may help in this examination. Next, the two lobes of the gland should be compared for size and texture using visual inspection, as well as manual or bimanual palpation. Finally, ask the patient to swallow to check for mobility of the gland; many clinicians find that having the patient swallow water helps this part of the examination. In a healthy state, the gland is mobile when swallowing occurs due its fascial encasement. Thus when the patient swallows, the gland moves superiorly, as does the whole larynx. [33]

A band of thyroid tissue, called the isthmus, bridges the lobes. Underneath the thyroid gland are the parathyroid glands (responsible for calcium balance) and the recurrent laryngeal nerves (innervations for the vocal cords). These later structures take on great significance during thyroid surgery when care must be exercised to avoid injury and resultant hypocalcaemia or permanent hoarseness, respectively. [27]

The thyroid is supplied with arterial blood from the superior thyroid artery, a branch of the external carotid artery, and the inferior thyroid artery, a branch of the thyrocervical trunk, and sometimes by the thyroidima artery, branching directly from the subclavian artery. The venous blood is drained via superior thyroid veins, draining in the internal jugular vein, and via inferior thyroid veins, draining via the plexus thyreoideus impar in the left brachiocephalic vein .[27]

Lymphatic drainage passes frequently the lateral deep cervical lymph nodes and the pre- and paratracheal lymph nodes. The gland is supplied by parasympathetic nerve input from the superior laryngeal nerve and the recurrent laryngeal nerve. [27]

The thyroid gland is responsible for the production of two hormones, thyroid hormone a polypeptide T4 and T3, both of which are iodinated derivatives of tyrosine and calcitonin. Calcitonin is secreted by parafollicular C cells and is involved in calcium homeostasis. Thyroid hormone is critical in regulating body metabolism, neurologic development, and numerous other body functions. Clinically, conditions affecting thyroid hormone levels are much more common. [27]

Thyroid disorders in which there is either over- or under-secretion of T4 and T3 are, however, common. The pituitary trophic hormone, TSH, stimulates thyroxine synthesis and release. The secretion of TSH is controlled by negative feedback by the thyroid hormones predominantly T4, which modulate the response of the pituitary to the hypothalamic hormone, thyrotrophin releasing hormone TRH.

Glucocorticoids, dopamine and somatostatin inhibit TSH secretion. The physiological significance of this is not known but it may be relevant to the disturbances of thyroid hormones that can occur in non-thyroidal illness. The major product of the thyroid gland is T4. Ten times less T3 is produced (the proportion may be greater in thyroid disease), most T3 approximately (80%) being derived from T4 by deiodination in peripheral tissues, particularly the liver, kidneys and muscle. T3 is (3-4 times) more potent than T4. Deiodination can also produce reverse triiodothyronine rT3, which is physiologically inactive. It is produced instead of T3 in starvation and many non-thyroidal illnesses, and the formation of either the active or the inactive

metabolite of T4 appears to play an important part in the control of energy metabolism. The anterior pituitary is also active in converting T4 to T3. It is thought that the pituitary senses thyroid hormone status through a change in the concentration of T3 due to deiodination within anterior pituitary cells. [34]

2.1.1.1 Prenatal development

In the embryo, at (3–4 weeks) of gestation, the thyroid gland appears as an epithelial proliferation in the floor of the pharynx at the base of the tongue between the tuberculum impar and the copula linguae at a point later indicated by the foramen cecum. The thyroid then descends in front of the pharyngeal gut as a bilobed diverticulum through the thyroglossal duct. Over the next few weeks, it migrates to the base of the neck, passing anterior to the hyoid bone. During migration, the thyroid remains connected to the tongue by a narrow canal, the thyroglossal duct. TRH and TSH start being secreted from the fetal hypothalamus and pituitary at 18-20 weeks of gestation, and fetal production of thyroxine T4 reach a clinically significant level at (18–20 weeks). [75] Fetal T3 remains low (less than 15 ng/dL) until (30 weeks) of gestation, and increases to (50 ng/dL) at term. Fetal self-sufficiency of thyroid hormones protects the fetus against e.g. brain development abnormalities caused by maternal hypothyroidism. [35]

However, preterm births can suffer neurodevelopmental disorders due to lack of maternal thyroid hormones due their own thyroid being insufficiently developed to meet their postnatal needs. [36]

The portion of the thyroid containing the parafollicular cells, responsible for the production of calcitonin, are derived from the neural crest. This is first seen as the ultimobranchial body, which joins the primordial thyroid gland during its descent to its final location in the anterior neck. Aberrations in prenatal development can cause various forms of thyroid dysgenesis. [36]

2.1.1.2 Histology of thyroid gland

At the microscopic level, there are three primary features of the thyroid: first discovered by Geoffary Websterson in 1664. [37]

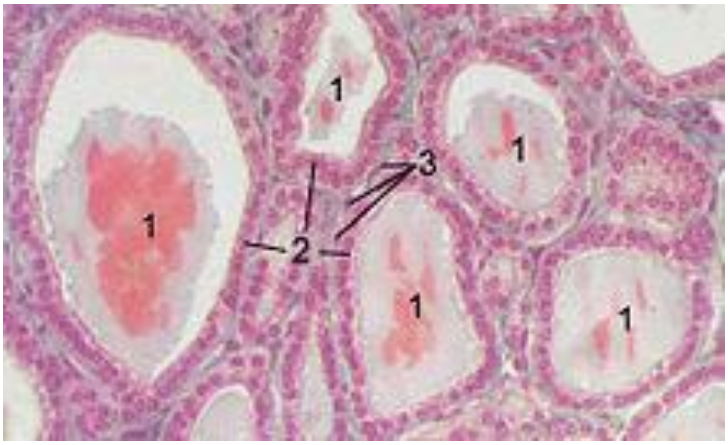

Figure (2.1) Histological section through the thyroid gland (1) follicles, (2) follicular epithelial cells, (3) endothelial cells [Bloom & Fawcett's Concise Histology]

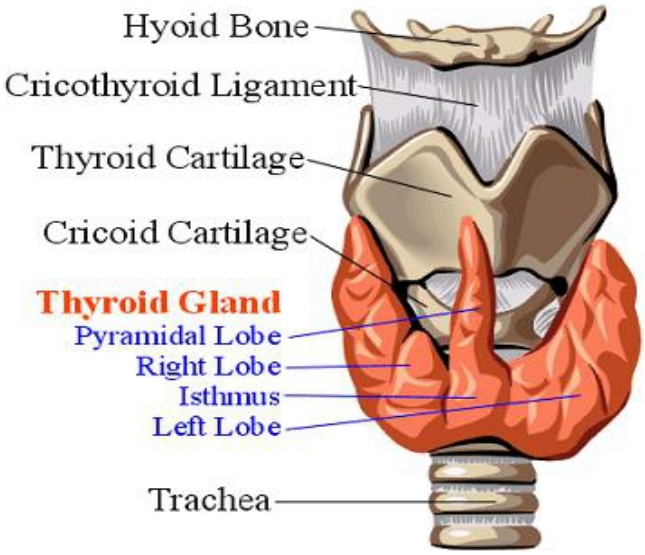

Figure (2.2) Anatomical view of Thyroid gland [Thyroid Anatomy (2015)]

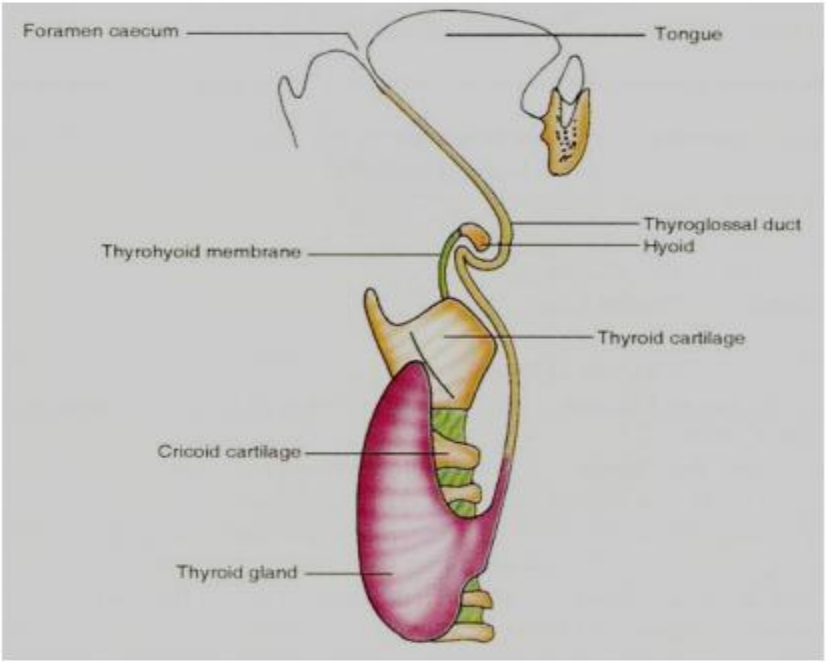

Figure (2.3) Embryonic development of thyroid gland [Anon, (2015)]

Table (2.1) histology of thyroid gland [37]

Feature	**Description**
Follicles	The thyroid is composed of spherical follicles that selectively absorb iodine as iodide ions, $\mathbf{I^-}$ from the blood for production of thyroid hormones, and also for storage of iodine in thyroglobulin. Twenty five percent of the body's iodide ions are in the thyroid gland. Inside the follicles, in a region called the follicular lumen, colloid serves as a reservoir of materials for thyroid hormone production and, to a lesser extent, acts as a reservoir for the hormones themselves. Colloid is rich in a protein called thyroglobulin
Follicular cells	The follicles are surrounded by a single layer of thyroid epithelial cells, which secrete T3 and T4. When the gland is

	not secreting T3 and T4 (inactive), the epithelial cells range from low columnar to cuboidal cells. When active, the epithelial cells become tall columnar cells.
Parafollicular cells (or "C cells")	Scattered among follicular cells and in spaces between the spherical follicles are another type of thyroid cell, parafollicular cells, which secrete calcitonin

2.1.2 Physiology of thyroid

The primary function of the thyroid is production of the hormones T3, T4 and calcitonin. Up to (80%) of the T4 is converted to T3. T3 is several times more powerful than T4, which is largely a prohormones, perhaps four or even (10-times) more active. [38]

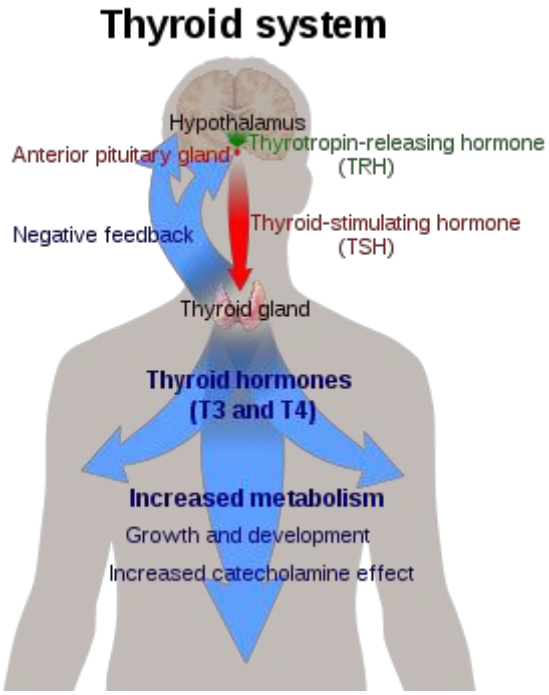

Figure (2.4): The system of the thyroid hormones T3 and T4 [the thyroid gland in Endocrinology]

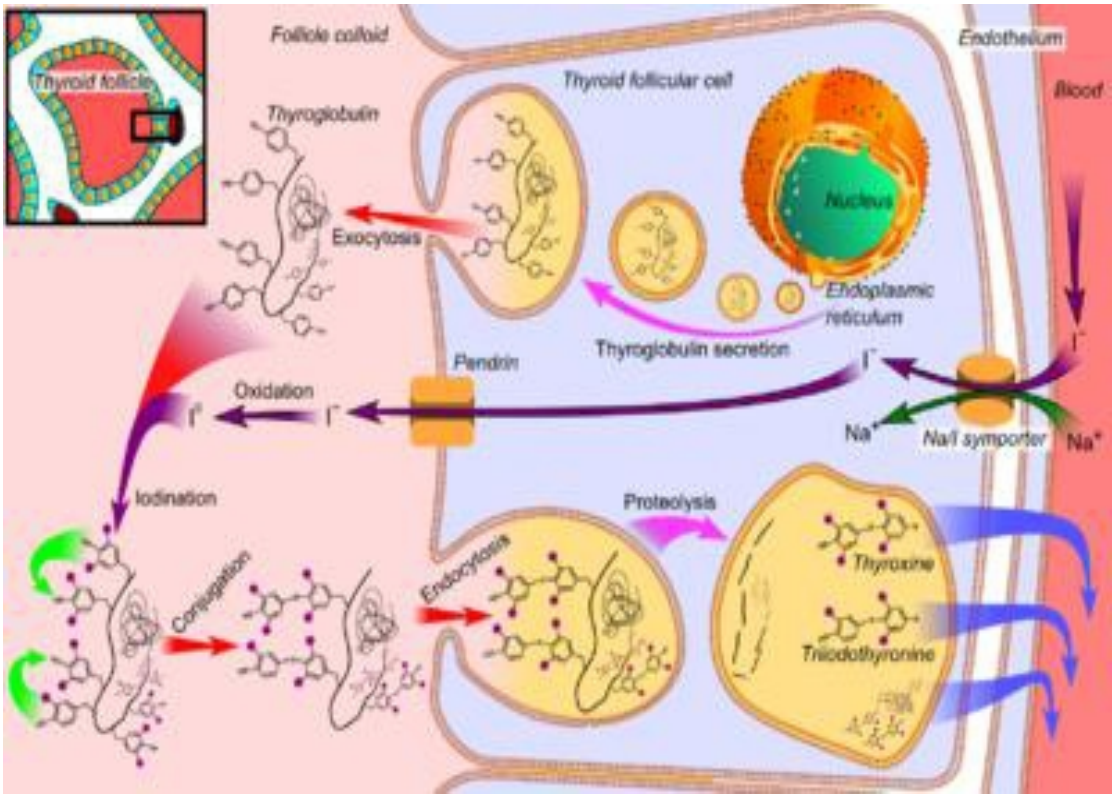

Figure (2.5) Thyroid hormone synthesis [Biochemistry 2002]

T3 and T4 production and action

Synthesis of the thyroid hormones, as seen on an individual thyroid follicular cell: [39]

- Thyroglobulin is synthesized in the rough endoplasmic reticulum and follows the secretory pathway to enter the colloid in the lumen of the thyroid follicle by exocytosis.

- Meanwhile, a Na/I symporter pumps iodide I^- actively into the cell, which previously has crossed the endothelium by largely unknown mechanisms.

- This iodide enters the follicular lumen from the cytoplasm by the transporter pendrin, in a purportedly passive manner. [40]

- In the colloid, iodide I^- is oxidized to iodine I^0 by an enzyme called thyroid peroxidase.

- Iodine I^0 is very reactive and iodinates the thyroglobulin at tyrosyl residues in its protein chain (in total containing approximately 120 tyrosyl residues).
- In conjugation, adjacent tyrosyl residues are paired together.
- The entire complex re-enters the follicular cell by endocytosis.
- Proteolysis by various proteases liberates thyroxine and triiodothyronine molecules, which enters the blood by largely unknown mechanisms.

T4 is synthesised by the follicular cells from free tyrosine and on the tyrosine residues of the protein called thyroglobulin Tg. Iodine is captured with the "iodine trap" by the hydrogen peroxide generated by the enzyme TPO **(41)** and linked to the (3' and 5') sites of the benzene ring of the tyrosine residues on Tg, and on free tyrosine. Upon stimulation by the TSH, the follicular cells reabsorb Tg and cleave the iodinated tyrosines from Tg in lysosomes, forming T4 and T3 in T3, one iodine atom is absent compared to T4, and releasing them into the blood. Deiodinase enzymes convert T4 to T3. **(42)** Thyroid hormone secreted from the gland is about (80-90% T4) and about (10-20% T3). (38)

Cells of the developing brain are a major target for the thyroid hormones T3, T4. Thyroid hormones play a particularly crucial role in brain maturation during fetal development. (43) A transport protein that seems to be important for T4 transport across the blood brain barrier the organic-anion transporting polypeptide 1C1, OATP1C1 has been identified. A second transport protein monocarboxylate 8, MCT8 is important for T3 transport across brain cell membranes. (44)

Non genomic actions of T4 are those that are not initiated by liganding of the hormone to intranuclear thyroid receptor. These may begin at the plasma membrane or within cytoplasm. Plasma membrane initiated actions begin at a receptor on the integrin alpha V beta3 that activates (ERK1/2). This

binding culminates in local membrane actions on ion transport systems such as the (Na^+/H^+) exchanger or complex cellular events including cell proliferation. These integrins are concentrated on cells of the vasculature and on some types of tumor cells, which in part explains the proangiogenic effects of iodothyronines and proliferative actions of thyroid hormone on some cancers including gliomas. T4 also acts on the mitochondrial genome via imported isoforms of nuclear thyroid receptors to affect several mitochondrial transcription factors. Regulation of actin polymerization by T4 is critical to cell migration in neurons and glial cells and is important to brain development. [45]

T3 can activate phosphatidylinositol 3-kinase by a mechanism that may be cytoplasmic in origin or may begin at integrin alpha V beta3.

In the blood, T4 and T3 are partially bound to TBG, transthyretin, and albumin. Only a very small fraction of the circulating hormone is fT4 (0.03%) and fT3 (0.3%). Only the free fraction has hormonal activity. As with the steroid hormones and retinoic acid, thyroid hormones cross the cell membrane and bind to intracellular receptors (α_1, α_2, β_1 and β_2), which act alone, in pairs or together with the retinoid X-receptor, RXR as transcription factors to modulate DNA transcription. [45]

The thyroid hormones T4 and T3 are released into the circulation by the thyroid gland under stimulation of TSH from the anterior pituitary gland. T3 is the active hormone which can bind to THRs in target cell nuclei. T4 must be de-iodinated to T3 to have nuclear effects; however, it is secreted in much larger amounts thought to be approximately (14 times) that of T3. Both thyroid hormones must be transported across lipid membranes into cells; this action is performed by thyroid hormone transporters. These are either specific for thyroid hormones or transport other peptides as well, and may be

specific to certain tissues or found throughout the body. Examples of specific thyroid hormone transporters include the MCT8 transporters [46] and OATP1C1. [47]

Once inside cells, thyroid hormones can be deiodinated by the D1, D2 and D3; these either activate the hormones by changing T4 into T3 D1 and D2, or effectively inactivate them by turning T4 into reverse T3 rT3 and T3 into T2, with both unable to produce effects D3. The deiodinases vary in their presence and activity within different tissues, and also in a temporal manner during development, allowing them to control thyroid hormone delivery to specific tissues. T3 then moves into the cell nucleus where it binds to TRs, resulting in a change in formation and binding of the receptor which binds to DNA and causes transcription of thyroid responsive genes. The TR often binds as a heterodimer with RXR and its action is also influenced by co-regulator proteins which can bind once T3 is bound to the receptor. These again vary between tissues, as does the TR, of which there are two main types and several isoforms. [47]

The production of T4, T3 is regulated by TSH. The thyroid and thyrotropes form a negative feedback loop: TSH production is suppressed when the T4 levels are high. [76] The TSH production itself is modulated by thyrotropin-releasing hormone TRH, which is produced by the hypothalamus and secreted at an increased rate in situations such as cold exposure to stimulate thermogenesis. TSH production is blunted by somatostatin SRIH, rising levels of glucocorticoids and sex hormones estrogen and testosterone, and excessively high blood iodide concentration.

An additional hormone produced by the thyroid contributes to the regulation of blood calcium levels. Parafollicular cells produce calcitonin in response to hypercalcemia. Calcitonin stimulates movement of calcium into bone, in

opposition to the effects of PTH. However, calcitonin seems far less essential than PTH, as calcium metabolism remains clinically normal after removal of the thyroid thyroidectomy, but not the parathyroids. [48]

2.1.2.1 Heritability of Thyroid Hormones

It has been recognized for some time that circulating TSH, fT4 and fT3 concentrations in euthyroid individuals have a much greater inter-individual than intra-individual variation. The width of the individual (95%) confidence interval for all three variables was approximately half that of the entire group. [49] As a result, although the population reference ranges for these parameters are wide, each individual appears to have their own set point within this. This has significant implications given that small changes in thyroid function, even within the population reference range, have been shown to have clinically detectable effects on phenotypes as varied as cholesterol, [50] mood [51] and longevity.[52]

2.1.2.1 .1Common Genetic Variation

Even unrelated human subjects share about (99.9%) of their genome. It has been estimated that 90% of the remaining variation is accounted for by approximately (10 million) common single nucleotide polymorphisms (SNPs), single base changes spread throughout the genome. These are very useful in studying gene-phenotype associations as they occur commonly in the general population, and may either cause changes in gene function themselves, or more frequently are markers of nearby elements that do. Due to publicly available databases such as that generated through the human genome project and the International HapMap [53] a considerable amount of information on the location, functionality and inheritance of these SNPs is freely available. Advancements in genetic technology have enabled genotyping to be performed rapidly and cheaply on large numbers of

subjects, further enhancing their usefulness. Methods used to identify associations between genes and thyroid phenotypes include candidate gene studies, genome-wide linkage studies, GWAS and whole genome sequencing. [54]

2.1.2.1 .2 Genetics of Thyroid Function

Whilst it is clear from heritability studies that a significant proportion of TSH, fT4, fT3 variation is genetically derived, the genes responsible for this remain largely undetermined, as discussed below. Thus far, polymorphisms within three genes have been shown to be associated with thyroid function in healthy subjects at genome-wide levels of significance: PDE8B, DIO1 and CAPZB. Furthermore, a polymorphism in the TSHR gene, whilst not associated with TSH at genome wide significance levels, has been shown to have associations with thyroid function in multiple studies in different populations. Many other possible candidates have not been replicated, whilst many genes which we would expect to influence thyroid function, such as the thyroid hormone receptors THRA and THRB and MCT8, have not shown associations. This may be because the genes have not been sufficiently finely mapped, studies have not had enough power, there may not be functional polymorphisms within these genes or these polymorphisms may not be compatible with life. [56]

Phosphodiesterase 8B

PDE8B found on chromosome 5 encodes a protein which catalyses the hydrolysis and inactivation of cAMP. performed a GWAS and discovered an A>G SNP, rs4704397 within this gene to be associated with circulating TSH concentrations, each copy of the rarer A allele conferring a mean increase of (0.13mU/L) TSH,[55] translating to an increase of (0.26 mU/L) for A homozygotes. The authors estimate this polymorphism is responsible for

approximately (2.3%) of TSH variation in their population. Whilst the original study did not contain fT4 or fT3 levels, three further studies have not shown any association of this SNP with fT4 or T3. [56, 57]

F-Actin-Capping Protein Subunit Beta (CAPZB)

Upstream of CAPZB another SNP, rs10917469, was discovered by GWAS to be associated with circulating TSH concentrations in healthy individuals. Found on chromosome 1, each of the rarer G allele is associated with lower mean TSH of approximately (0.16 mU/L) and similar to PDE8B is not associated with fT4 or fT3 levels, suggesting it alters pituitary-thyroid set points. It is estimated this SNP is responsible for about (1.3%) of the total variation of TSH. The mechanism by which it affects TSH is unknown. The F-actin capping protein subunit beta binds to the fast growing end of the actin filament, blocking the exchange of subunits and regulating growth. [58]

TSH Receptor

Several smaller studies have independently found associations between TSHr, SNPs and TSH concentrations, and some have shown associations with clinical phenotypes, suggesting this is a real association. However, GWAS have not shown SNPs in TSHr to be associated with TSH concentrations at a high level of significance, which raises doubts as to the validity of these associations. [55, 58]

This SNP is not associated with fT4 or fT3 levels and is thought to influence pituitary - thyroid axis set points by changing the sensitivity of the TSHr to TSH. [59]

2.1.3 Iodine

Iodine is a fundamental micronutrient for the organism which should be regularly administered through foods. Its function is essential for the synthesis of thyroid hormones which in turn act on the different organs and

systems of the organism especially in the development of the CNS from the earliest stages of embryonic and fetal development. [60]

The ingestion of iodine depends on the type of foods consumed, their origin and preparation. Depending on the geographical area, products from the earth may have scarce iodine content. However, foods of marine origin are rich in this micronutrient. [60] In addition, it should be taken into account that foods lose iodine during their preparation: (20%) is lost on frying, (23%) on baking and (58%) on boiling. [61] Some studies have shown that the iodine content of cows' milk differs according to what the animals are fed. [62]

All these factors and alimentary habits make it difficult for the daily iodine requirements of the population to be covered through diet. Moreover, iodine is not stored in the body and must therefore be continually replenished. In normal conditions there is equilibrium between iodine intake and urinary elimination, and determination of iodine in urine constitutes a good indicator of iodine intake, [63] with assessment of ioduria in a casual urine sample providing adequate information on the nutritional status of iodine. [64]

During pregnancy there is an increase in thyroid hormone requirements due to the physiological modifications produced in response to the metabolic demands of pregnancy. This increase can only be achieved by a proportional increase in hormone production which directly depends on the availability of iodine in the diet. Moreover, gestation produces a physiological increase in the elimination of iodine in the urine because of a rise in glomerular filtration. In cases with an underlying deficit in iodine these modifications of pregnancy may not be compensated leading to failure of the mechanisms of adaptation. It is therefore very important to increase iodine intake from the beginning of gestation and even beforehand if possible, similar to the recommendations of supplementation of folic acid. The thyroid hormones

available for embryonic and fetal tissue during the first trimester of gestation depend exclusively on maternal hormones and thus, a deficit in maternal iodine can have negative repercussions on prenatal development. [60]

According to the WHO together with the UNICEF and the ICCIDD the iodine needs of pregnant women have been established as (200µg/L/day).

The ICCIDD has recently raised this recommendation to (250-300µg/L/day). [63]

Maternal iodine intake may be calculated by the determination of ioduria taking into account that the factor of dilution in urine is greater in pregnant women than in the remaining population. The value of ioduria indicating optimum iodine intake during gestation should be between (150 and 230µg/L). [65] Studies carried out in different European countries such as France, England, Germany, Switzerland, Ireland and Hungary have demonstrated the highly variable values of iodine deficiency values less than (150 µg/L) in pregnant women, from (3.5%) in England to (57.1%) in Hungary. [66, 67]

Another aspect to consider within hygienic dietetic habits of pregnant women is smoking and the repercussion this may have on maternal thyroid function. Smoking is considered a goitrogenic substance since it inhibits the absorption of iodine during both gestation and the period of lactation. [67]

Smoking during gestation is associated with changes in the levels of thyroid function in both the mother and the fetus. The concentration of TSH in the mother (in the first and third trimester of gestation) and in the blood of the umbilical cord are lower and the T3 levels are higher which may trigger adverse effects for both. [56, 69] It has also been reported that smoking during the period of lactation increases the risk of iodine deficiency which may lead to brain damage in the lactating child. [67]

Iodine deficiency is not only a problem in developing countries but also affects most of the industrialized countries to a greater or lesser degree. It has currently been estimated that half of the population lives in areas in which there is a risk of having disorders due to iodine deficiency such as what occurs in several European countries such as Germany, Belgium, Denmark, Spain, France, Greece, Ireland and Italy. Zones with endemic goiter or some other alterations related to iodine deficiency have been detected in Spain. [70, 71]

Until several years ago the fundamental problem of iodine deficiency lay in endemic goiter but in the last decades studies have demonstrated a wide spectrum of disorders caused by iodine deficiency during pregnancy such as an increase in the number of abortions and dead fetuses, an increase in neonatal morbimortality and hearing defects in infants, [72] a reduction in intellectual capacity and growth, congenital abnormalities with permanent neuromotor damage [73, 74] as well as the attention deficit syndrome and hyperactivity. [77, 78] According to the WHO, lack of iodine is the most frequent cause of mental retardation and irreversible brain lesions in the world. [63]

2.1.3 .1 Significance of iodine

In areas of the world where iodine is lacking in the diet, the thyroid gland can become considerably enlarged, a condition called endemic goiter. Pregnant women on a diet that is severely deficient of iodine can give birth to infants with congenital hypothyroidism, manifesting in problems of physical growth and development as well as brain development (endemic cretinism).

The use of iodized salt is an efficient way to add iodine to the diet. It has eliminated endemic cretinism in most developed countries, and some

governments have made the iodination of flour, cooking oil, and salt mandatory. Potassium iodide and sodium iodide are typically used forms of supplemental iodine. As with most substances, either too much or too little can cause problems. Recent studies on some populations are showing that excess iodine intake could cause an increased prevalence of autoimmune thyroid disease, resulting in permanent hypothyroidism. [79]

2.1.3 .2 Iodine as an Essential Element for Thyroid Hormone

Iodine; average atomic weight 126.9 is a trace chemical element primarily found in oceans as the highly water soluble iodide ion (I^-). [80] In humans, TH is important for normal growth and differentiation of cells, fetal growth, nervous system development, bone formation, reproductive tract development. [81]

The synthesis of mammalian thyroid hormone requires the transport of (I) into thyroid cells. The sodium/iodide (Na^+/I^-) symporter NIS, an (87-kDa) transmembrane protein on the basolateral membrane of thyroid follicular cells, pumps two Na^+ and one I^- from the bloodstream into cells. I^- is then transported across the apical membrane into the colloid of the follicular lumen by a Cl^-/I^- transporter, thought to be the pendrin PDS protein. [82, 83] Tg, a large glycoprotein precursor of thyroid hormone, is also found in the colloid, following synthesis in the endoplasmic reticulum. In the colloid, the enzyme thyroid peroxidase catalyzes the oxidation of I^- to I^+ and iodination of the tyrosyl residues of Tg molecules to generate MIT and DIT. Via conjugation, either two adjacent DIT particles are paired to produce T4 or one MIT and one DIT are paired to produce T3, which has three iodine atoms, one less iodine atom than T4. Iodinated Tg is reabsorbed by the action of TSH into thyroid cells, where it is digested by proteases to release T4 and T3 from the backbone of its protein chain into circulation. [84]

2.1.3 .3 Global Prevention and Elimination of Iodine Deficiency

Thyroid hormone plays a central role in the intermediary metabolism of virtually all tissues and is of fundamental importance for the development of the CNS in the fetus and the newborn. **(85)** Therefore, iodine deficiency due to a lack of dietary iodine, typically seen in remote inland areas, where no marine foods are available, became a leading cause of developmental delays, mental retardation, endemic goiter and many other health problems. (86)

Fortunately, IDD are a preventable public health problem with a simple and inexpensive solution. Iodine supplementation, such as USI, was introduced in order to prevent and eliminate IDD. USI is a global strategy recommended by the UNICEF, WHO in 1994 to ensure adequate dietary iodine through the addition of potassium iodate to salt. Substantial progress has been made by such global efforts to control IDD. Over the past decade, the number of iodine deficient countries has fallen from (54 to 30); the number of iodine-sufficient countries has increased from (67 to 112); and approximately (70%) of households worldwide have access to adequate iodized salt. (87, 88)

2.1.3 .4 Iodine excess as another Concern

Iodine supplementation must be carefully monitored to ensure adequate iodine intake while avoiding iodine excess. WHO data show that adequate or excessive iodine intake has been observed in over 30 countries. (89, 88)

Investigations of these instances have identified numerous factors, including high levels of salt iodization and overlapping iodine supplementation, as well as routine consumption of particular iodine rich foods. Risks involved in iodine excess, such as hypothyroidism, hyperthyroidism, cancers, autoimmune thyroid disease (ATD), etc., have drawn more concerns than before, as iodine excess is an increasingly more frequent occurrence. (87, 88)

2.1.3 .5 Iodine an environmental risk factor for Auto immune thyroid disease

Regional dietary sources that are naturally rich in iodine can contribute to iodine excess in some countries or regions. In Asian countries, such as Japan and Korea, seaweed is a popular food, especially in coastal areas. Some edible seaweed is rich in iodine [90, 91] and has been identified as a unique risk factor for excess iodine in these areas. Cases of iodine excess or even iodine toxicity due to overindulging in seaweed have been reported. Investigations also indicate that this dietary pattern is associated with high morbidity of thyroid disorders, including goiter, thyroid cancers and ATD, in coastal areas of Japan. [92, 93]

In some areas of China, drinking water with high levels of iodine has been reported and identified as the key contributor to iodine excess. [94, 95] Such iodine rich drinking water has also been found in Somalia, [96] Saharawi [97] and Europe. [98] The application of iodine containing water purification tablets is another source of excess iodine exposure from drinking water. [99, 100] In the areas where residents ingest high iodine drinking water, the iodine content of edible salt should be lowered accordingly to avoid iodine excess. [101] Moreover, due to the high iodine content in animal feed (including grass) and/or the use of iodophor cleaners for milk cans, milk and dairy products can also be rich in iodine and become a potential contributor to excess iodine in Western countries, where milk and dairy products are a major part of the diet. [102, 103] Thus, when iodized salt alone is supposed to provide enough iodine, the additional routine consumption of other iodine rich foods or drinking water can lead to chronic iodine excess in the body. Iodine excess has been observed much more frequently since iodide supplementation by USI was initiated.

Worldwide, iodinated salt, as well as processed foods containing iodized salt (e.g., bread, milk and snack foods), are the most extensive dietary sources for iodine today. However, due to the variable iodine content in edible salt, poor monitoring of production and social iodine status, salt iodine sometimes can exceed the adequate level for a particular community. A number of reports have associated high levels or overconsumption of iodized salt in food with iodine excess and thyroid disorders in Mexico, [104] Somalia, [105] China, [101, 106] Bulgaria, [107] Brazil, [108] Sri Lanka [109] and African countries. [110, 111] Although less common, non-dietary sources of iodine sometimes contain levels that are hundreds to thousands of times higher than in the diet. Excess iodine ingestion from nutritional supplements, such as multivitamin tablets, often goes unrecognized.

However, investigations in the USA showed that the actual iodine content in (60) randomly selected iodine - containing multivitamin brands varied from (11 to 610µg per daily dose), including (15) brands with higher iodine content than was stated on the labels. [112] In addition, excess iodine ingestion from maternal nutritional supplements during pregnancy has reportedly led to congenital hypothyroidism. [113]

Among iodine-rich medications, amiodarone, a drug commonly used to treat ventricular and supraventricular tachyarrhythmias, contains (37%) iodine. Thus, one tablet can contain several hundred times the recommended daily intake of iodine. Moreover, amiodarone has a long half-life and easily accumulates in vivo. Therefore, amiodarone has been shown to be the most common medication source of excess iodine and a risk factor for medication-induced thyroid disorders. [114, 115]

Another common source of excess iodine in medical practices is iodinated contrast agents used for diagnostic radiology. A single dose of iodinated

contrast usually contains much more iodine (hundreds of thousands of times higher) than the recommended daily dose. Iodine levels in the body will remain elevated after digestion of iodinated contrast, and it can take more than one month for iodine levels to be normalized following exposure. [116]

2.1.3.6 Excess Iodine is an environmental factor for autoimmune thyroiditis

Although the mechanisms are not fully elucidated, excess iodine is a well-recognized environmental factor for ATD in autoimmune prone individuals, particularly AIT, which is characterized by lymphocytic infiltration of the thyroid gland with the development of thyroid autoantibodies and primary hypothyroidism. [117]

Large bodies of epidemiological and clinical data from countries and regions have associated high iodine levels with the development of thyroid autoantibodies and thyroid dysfunctions, including goiter, hypothyroidism, cancers and the morbidity of AIT. [118, 119, 120]

However, the mechanism underlying the use of iodine to treat Graves' disease may involve more than the negative feedback effect on iodine organification and thyroid hormone synthesis. In vivo and in vitro evidence shows that even a short period of administrating a high concentration of iodine could reduce the expression of major histocompatibility complex MHC class I and class II in the thyrocytes of Graves' patients. [121] The exact mechanism is unknown, but probably involves nuclear factor κB-mediated gene expression. [92] Iodine depletion has also been associated with increased MHC expression in nontoxic goiters, indicating another potential effect of iodine deficiency. [122]

2.1.4 Disorders of the thyroid

Hypothyroidism and hyperthyroidism are common diseases, which are treated with hormone replacement or antithyroid drugs, respectively. Applied therapies are targeted at adjusting the serum thyroid stimulating hormone TSH concentration to values within the reference range. [123]

2.1.4 .1 Hyperthyroidism

Hyperthyroidism, or overactive thyroid, is defined as an overproduction of the thyroid hormones T3 and T4. This condition is most commonly caused by the development of GD, an autoimmune disease in which anomalous antibodies stimulate the thyroid to secrete excessive quantities of TH. The disease can progress to the formation of a toxic goitre as a result of thyroid growth in response to a lack of negative feedback mechanisms. It presents with symptoms such as a thyroid goitre, protruding eyes (Exophthalmos), palpitations, excess sweating, diarrhea, weight loss, muscle weakness and unusual sensitivity to heat. The appetite is often increased. [124]

Beta blockers are used to decrease symptoms of hyperthyroidism such as increased heart rate, tremors, anxiety and heart palpitations, and anti thyroid drugs are used to decrease the production of thyroid hormones, in particular, in the case of Graves' disease. These medications take several months to take full effect and have side-effects such as skin rash or a drop in white blood cell count. These drugs involve frequent dosing (often one pill every 8 hours) and often require frequent doctor visits and blood tests to monitor the treatment, and may sometimes lose effectiveness over time. Due to the side effect, and inconvenience of such drug regimens, some patients choose to undergo radioactive I^{-131} treatment. Radioactive iodine is administered in order to destroy a portion of or the entire thyroid gland, since the radioactive iodine is selectively taken up by the gland and gradually destroys the cells of

the gland. Alternatively, the gland may be partially or entirely removed surgically, though iodine treatment is usually preferred since the surgery is invasive and carries a risk of damage to the parathyroid glands or the nerves controlling the vocal cords. If the entire thyroid gland is removed, hypothyroidism is results. [125]

2.1.4 .2 Hypothyroidism

Hypothyroidism is the underproduction of the T3& T4, and may occur as a result of :-

- Congenital thyroid abnormalities
- Autoimmune disorders such as Hashimoto's thyroiditis
- Iodine deficiency
- The removal of the thyroid following surgery to treat severe hyperthyroidism and/or thyroid cancer

Typical symptoms are abnormal weight gain, baldness, cold intolerance, and bradycardia, [126] fatigue, dry skin, and constipation, and hoarseness, dyspnea on exertion, cognitive dysfunction, hair loss, and weight gain are reported. [27] Hypothyroidism is treated with hormone replacement therapy, such as levothyroxine, which is typically required for the rest of the patient's life. Thyroid hormone treatment is given under the care of a physician and may take a few weeks to become effective. [126]

This condition is diagnosed by a low fT4 level (in primary or central hypothyroidism) and/ or a high TSH (in primary hypothyroidism). On physical examination, patients with severe hypothyroidism may have low body temperature, slow movements, bradycardia, delay in the relaxation phase of deep tendon reflexes, yellow discoloration of the skin (from hypercarotenemia), hair loss, diastolic hypertension, pleural and pericardial effusions, menstrual irregularities, and periorbital puffiness. [27]

Hypothyroidism can lead to a variety of other abnormalities. Hypothyroidism, through inappropriate levels of antidiuretic hormone, can lead to hyponatremia. Significant hypothyroidism can also lead to myopathy and high levels of creatine phosphokinase (CPK). Anemia can also be seen in hypothyroidism. The etiology of the anemia can be a result either of lower demand oxygen carrying capacity or through associated autoimmune pernicious anemia. Hypothyroidism can also lead to hyperlipidemia, especially when the TSH is greater than (10mU/L). One study documented that (4.2%) of patients with hyperlipidemia had hypothyroidism. Another study documented that more than one-half of patients with hypothyroidism had hyperlipidemia. In all of the conditions listed above (hyponatremia, unexplained high CPK levels, anemia, hyperlipidemia), it is prudent to evaluate for hypothyroidism as a secondary cause. Hypothyroidism can be divided into primary, secondary, or tertiary disease, dependent on whether the defect is located in the thyroid gland, pituitary gland, hypothalamus, respectively. The most common cause of hypothyroidism in developed countries is chronic lymphocytic thyroiditis, or HT. This is an autoimmune disease of the thyroid gland, which is often associated with enlargement of the thyroid gland (goiter). TPOAb testing will be positive in (80-90%) of patients with chronic lymphocytic thyroiditis. Other common causes of hypothyroidism include iodine deficiency, thyroid surgery, and radioactive iodine treatment. Certain drugs can cause hypothyroidism. Occasionally, patients will experience transient hypothyroidism associated with inflammation of the thyroid gland. Example of transient hypothyroidism includes recovery from nonthyroidal illness and the hypothyroid phase of one of several forms of subacute thyroiditis (painful thyroiditis, postpartum thyroiditis, and painless thyroiditis). [(127)]

Hypothyroidism is common; (5-15%) of women older than age (65) have this condition. For this reason, several organizations have recommended routine periodic assessment of thyroid function in women. [27]

Neonatal primary hypothyroidism may be caused by a congenitally absent, atrophic, or dysfunctional thyroid gland, a disorder that occurs once in every (3500 live births). As mentioned earlier, thyroid function is necessary for neurological development; therefore, untreated neonatal hypothyroidism results in profound impairment of growth and mental development. This disorder was formerly termed cretinism. [128]

Hypothyroidism is treated with thyroid hormone replacement therapy. Levothyroxine T4 is the treatment of choice. In primary hypothyroidism, the goal of therapy is to achieve a normal TSH. If hypothyroidism is of pituitary or hypothalamic origin (secondary or tertiary hypothyroidism), TSH levels will not be useful in managing the condition and a midnormal fT4 level becomes the target of therapy. [27]

There are many causes of primary hypothyroidism but hypothyroidism can also occur secondarily to decreased trophic stimulation both in hypopituitarism and in hypothalamic disease. It is, however, very rare for patients with pituitary failure to present with clinical features of hypothyroidism alone. The commonest cause of hypothyroidism is atrophic myxoedema, the result of autoimmune destruction of the gland. The clinical manifestations are variable and may result in the patient being referred to almost any specialist department in a hospital. [34]

Clinical diagnosis is confirmed by the finding of a high plasma TSH concentration (unless the condition is secondary to hypopituitarism) and low T4 concentration. Measurement of T3 is of no value in the diagnosis of hypothyroidism. Levothyroxine has a half-life of approximately (7 days),

when doses of thyroid hormone are changed. It is important to wait at least five half-lives before rechecking thyroid function tests to achieve a new steady state. It is usual to start with a small dose (50lag/day) and increase this at (4 - 6 week) intervals on the basis of the results of thyroid function tests. In the elderly and patients with ischaemic heart disease, a lower starting dose (25lag/day) should be used. There is a risk that the increase in metabolic rate and demand for oxygen prompted by hormone replacement may precipitate angina or myocardial infarction. Triiodothyronine has a more rapid onset of action and is preferable in the initial treatment of patients in myxoedema coma. In the laboratory, thyroid hormone replacement can be monitored by measuring plasma TSH and, if this is abnormal, TT4 concentrations TT3 if the patient is being treated with T3. Ideally, the replacement dosage should be sufficient to maintain TSH within the reference range. Too high a concentration indicates inadequate replacement; a suppressed TSH suggests excessive replacement and a risk of causing atrial fibrillation and, possible osteoporosis. In patients treated with T4, the plasma concentrations associated with a clinically euthyroid state are generally somewhat higher than the normal euthyroid range, because there is no contribution to endogenous hormone activity by secreted T3. If the dosage is changed, the results of thyroid function tests may not reach a new steady state for some time. The expected fall in TSH concentration lags behind the increase in that of fT4 when treatment is started, and, if the TSH has been suppressed because of over- replacement, months may elapse before normal thyrotrophic responsiveness to T4 is regained. Compliance with and the adequacy of treatment should be checked annually by measurements of TSH and, if this is abnormal, T4. Usually non-compliant patients who take their tablets regularly for a few days before a blood test

will be revealed by their having a raised TSH but a normal or even elevated if T4. Occasional patients with hypothyroidism present as an emergency with stupor and hypothermia. This 'myxoedema coma' has a high mortality. In addition to thyroid hormone replacement, usually with T3, possible coexistent adrenal insufficiency must be treated with hydrocortisone and appropriate measures taken to treat any infection, heart failure or electrolyte imbalance and to restore body temperature to normal. [34]

Table (2.2): Common Signs and Symptoms of Hypothyroidism: [1]

Sign or symptom	Affected patients (%)
Weakness	99
Skin changes (dry or coarse skin)	97
Lethargy	91
Slow speech	91
Eyelid edema	90
Cold sensation	89
Decreased sweating	89
Cold skin	83
Thick tongue	82
Facial edema	79
Coarse hair	76
Skin pallor	67
Forgetfulness	66
Constipation	61

Clinical biochemistry laboratories undertake large numbers of tests of thyroid function. To simplify their procedures, many adopt the approach of measuring TSH as a first-line test of thyroid function, adding other tests as required, for example if the concentration of TSH is found to be outside the

euthyroid reference range or if there is a strong suspicion that thyroid dysfunction is secondary to pituitary disease (though this is far less common than primary thyroid dysfunction). A combination of tests may also be required to assess patients being treated for thyroid disease, particularly in the early stages. It should be noted that immunometric assays such as is used for TSH are subject to interference by naturally occurring heterophilic antibodies against the monoclonal antibodies used in the assay; such interference occurs only infrequently, but can give rise to apparently high results. When the results of assays do not accord with those expected from the patient's clinical condition, it may be prudent to repeat them using an alternative method. [34]

2.1.4 .3 Thyroiditis

There are two types of thyroiditis where initially hyperthyroidism presents which is followed by a period of hypothyroidism; the overproduction of T3 and T4 followed by the underproduction of T3 and T4. These are HT and postpartum thyroiditis.

- HT or Hashimoto's Disease is an autoimmune disorder whereby the body’s own immune system reacts with the thyroid tissues in an attempt to destroy it. At the beginning, the gland may be overactive, and then becomes underactive as the gland is damaged resulting in too little thyroid hormone production or hypothyroidism. Some patients may experience "swings" in hormone levels that can progress rapidly from hyper – to - hypothyroid (sometimes mistaken as severe mood swings, or even being bipolar, before the proper clinical diagnosis is made). Some patients may experience these "swings" over a longer period of time, over days or weeks or even months. Hashimoto's is more common in females than males, usually appearing after the age of 30, and tends to run in families, meaning it can be seen as a

genetic disease. Also more common in individuals with Hashimoto's thyroiditis are DM type I and celiac disease. [130]

- Postpartum thyroiditis occurs in some females following the birth of a child. After delivery, the gland becomes inflamed and the condition initially presents with overactivity of the gland followed by underactivity. In some cases, the gland may recover with time and resume its functions. In others it may not. The etiology is not always known, but can sometimes be attributed to autoimmunity, such as HT or Graves' disease.

There are other disorders that cause inflammation of the thyroid, and these include subacute thyroiditis, acute thyroiditis, silent thyroiditis and Riedel's thyroiditis. [131]

HT is the most common autoimmune thyroid disease and the most common cause of hypothyroidism. [132] Epidemiological and histological data indicate that thyroid cancer TC frequently occurs in the context of one of the most common autoimmune thyroid diseases, HT, and that TC is frequently infiltrated by inflammatory immune cells. [133]

HT is characterized by infiltration of the thyroid gland by inflammatory cells. This often leads to hypothyroidism due to destruction and eventual fibrous replacement of the parenchymal tissue. The relationship between HT and papillary carcinoma PC was first proposed in 1955. Since this initial description, the association between the diseases has been repeatedly reported and highly debated in the literature and remains controversial. A relationship between chronic inflammation and cancer was first proposed by Virchow 1863 and has been sustained by clinical and epidemiological evidence. [134]

The most compelling evidence is the association between

i) Intestinal chronic inflammatory diseases (Crohn's disease and ulcerative rectocolitis) and adenocarcinoma of the colon;

ii) Chronic HBV or HCV hepatitis and liver carcinoma;

iii) Heliobacter pylori-induced chronic gastritis and gastric carcinoma;

iv) Asbestosis and mesothelioma;

v) Chronic obstructive pulmonary disease and lung cancer;

vi) Scleroderma and carcinoma of the breast and lung. [133, 135]

2.1.4 .4Autoimmune thyroid disease

ATD is the most common autoimmune condition, affecting approximately (2%) of the female population and (0.2%) of the male population. [136] Its overall prevalence peaks in adulthood; it is also the most common etiology of acquired thyroid dysfunction in pediatrics. It is more common in females and usually occurs in early to mid - puberty. Optimal quantities of thyroid hormone are critical to neurodevelopment and growth. The paediatrician can often recognize thyroid dysfunction in its early stages, by maintaining an appropriate index of suspicion. This review will analyze current opinions and options regarding the etiology, evaluation, diagnosis, treatment, and prognosis of ATDs in children. [137, 138]

2.1.4.4.1 Etiology: Autoimmune thyroid disease arises due to complex interactions

Between environmental and genetic factors, that is yet to be completely defined. ATD is multifactorial in that a genetic predisposition combines with environmental risk factors to promote disease.

Early evidence that ATD has a hereditary component stems from studies of familial aggregation. Several studies of young people with ATDs showed a definite genetic propensity for thyroid autoimmunity to run in families. [139]

Further evidence of the genetic control of ATDs comes from the observation of twins. Monozygotic twins show a higher concordance rate of disease than dizygotic twins. However, even with identical twins the concordance rate is only about (50%), emphasizing that other important factors, such as the environment, play a role in disease pathogenesis. [140, 141] The identified ATDs susceptibility genes can be divided into two broad groups:

(1) Immune modulating genes.

(2) Thyroid specific genes.

The immune modulating genes so far identified are:

HLA-DR, CTLA-4, CD40, and PTPN22, the CTLA-4 gene is a major negative regulator of T-cell activation. [142] CTLA-4 activation has been shown to suppress several experimental autoimmune diseases. CD40 **[143]** is expressed primarily on B cells and other APCs and plays a fundamental role in (B-cell) activation inducing, upon ligation, (B-cell) proliferation, immunoglobulin class switching, antibody secretion, and generation of memory cells. The lymphoid tyrosine phosphatase, encoded by the PTPN22 gene, like CTLA-4, is a powerful inhibitor of (T-cell) activation. [144]

Among the nongenetic factors postulated to precipitate ATDs are iodine [145, 146] and medications such as amiodarone [147] and interferon α, [148] infections, smoking, and stress. Amiodarone is a benzofuranic derivative iodine-rich drug widely used for the treatment of tachyarrhythmias. It often causes changes in thyroid function tests (typically an increase in serum T4 and rT3 and a decrease in serum T3 concentrations), mainly related to the inhibition of 5-deiodinase activity. In (14–18%) of amiodarone-treated patients, there is overt thyroid dysfunction, either amiodarone-induced thyrotoxicosis AIT or amiodarone-induced hypothyroidism AIH. Both AIT and AIH may develop either in apparently normal thyroid glands or in glands with

preexisting, clinically silent abnormalities. Preexisting Hashimoto's thyroiditis is a definite risk factor for the occurrence of AIH. The pathogenesis of iodine-induced AIH is related to a failure to escape from the acute Wolff - Chaikoff effect due to defects in thyroid hormonogenesis and, in patients with positive thyroid autoantibody tests, to concomitant Hashimoto's thyroiditis. AIT is primarily related to excess iodine-induced thyroid hormone synthesis in an abnormal thyroid gland (type I AIT) or to amiodarone- related destructive thyroiditis (type II AIT), but mixed forms frequently exist. [147] A few studies have shown seasonality [149, 150] and geographic variation [151] in the incidence of GD, adding evidence that infectious agents may trigger ATDs. Moreover several infectious agents have been implicated including Yersinia enterocolitica, [152, 153] Coxsackie B virus**,** [154] retroviruses [155, 156] and Helicobacter pylori. [157] By now, the strongest association of ATDs with an infectious agent is with HCV. [158] In most studies examining the frequency of thyroid disorders in HCV patients, approximately (10%) of the patients had positive autoantibodies prior to initiation of interferon therapy. [159, 160] All studies on HCV infection and thyroid autoimmunity demonstrated a significantly increase in the risk of ATDs in HCV patients. [161] Two main theories have been proposed for the induction of autoimmunity by infectious agents:

(1) The "molecular mimicry" theory suggests that sequence similarities between viral or bacterial proteins and self proteins can induce a cross-over immune response to self antigens. [162]

(2) The "bystander activation" theory proposes that viral infection of a certain tissue can induce local inflammation and cytokine release, resulting in activation of autoreactive (T cells), that were suppressed by peripheral regulatory mechanisms. [163]

2.1.4.4.2 Autoimmune Thyroiditis (AT)

The childhood prevalence of chronic autoimmune thyroiditis AT peaks in early to mid-puberty, and a female preponderance of (2:1) have been reported. [165] Presentation is rare under the age of (3 yrs), but cases have been described even in infancy. [164]

In 1912, Hashimoto described four women with goiter and the apparent transformation of thyroid into lymphoid tissue (struma lymphomatosa). These patients comprise the first report of Hashimoto's disease, which we now recognize as a form of AT. Improvements in the measurement of circulating autoantibodies and ultrasonography have obviated the need for biopsy in the diagnosis of AT. The term thyroiditis is defined as evidence of "intrathyroidal lymphocytic infiltration" with or without follicular damage. Two types of AT (also defined as chronic lymphocytic thyroiditis) are causes of persistent hypothyroidism: HT (goitrous form) and atrophic thyroiditis (non goitrous form). Both are characterized by circulating thyroid autoantibodies and varying degrees of thyroid dysfunction, differing only by the presence or absence of goiter. Transient thyroiditis seems to be a variant presentation of AT. It is characterized by an autoimmune-mediated lymphocytic inflammation of the thyroid gland resulting in a destructive thyroiditis with release of thyroid hormone and transient hyperthyroidism, frequently followed by a hypothyroid phase and full recovery. The condition is particularly common in the postpartum period, but it has been observed also in children. The term chronic AT does not include subacute thyroiditis.

2.1.4.4.2.1 Pathophysiology

The activation of (CD4) helper) T- lymphocytes specific for thyroid antigens is believed to be the first step in pathogenesis. Once activated, self-reactive CD4 T cells recruit cytotoxic CD8 T cells as well as autoreactive B cells into

the thyroid. The three main targets of thyroid antibodies are Tg, TPO, and the TSHr. TPOAb have been shown to inhibit the activity of the enzyme in vitro, but direct cytotoxicity by CD8 T cells is believed to be the main mechanism of hypothyroidism in vivo. TSHrAb of the blocking type may contribute to hypothyroidism in a minority of adult patients with the atrophic form of AT, but this has not been proven in children. Histologically, goitrous AT is characterized by diffuse lymphocytic infiltration with occasional germinal centers. Thyroid follicles may be reduced in size and contain sparse colloid. Individual thyroid cells are often enlarged with oxyphilic cytoplasm (usually defined Hurthle cells). In contrast, the gland of atrophic AT is small, with lymphocytic infiltration and fibrous replacement of the parenchyma. [166]

2.1.4.4.2.2 Clinical Aspects

AT is usually suspected in the presence of goiter, even in the absence of signs and symptoms of thyroid dysfunction. It may also be diagnosed incidentally during medical checkups, screening evaluation of children with growth defects, or follow-up of children with associated diseases, mainly Down syndrome, Turner syndrome, DM type 1, and celiac disease. [167, 168]

Symptoms and signs of overt hypothyroidism

Goiter, Poor linear growth with increased weight for height, Bone maturation delay, Pubertal disorders (pubertal delay or pseudoprecocious puberty), Irregular menstrual periods, Lethargy and/or impaired school performance, Fatigue, Bradycardia and decreased cardiac output, Constipation, Cold intolerance, Hypothermia, Fluid retention and weight gain (due to impaired renal free water clearance), Puffness of the face, Dry skin, Increased body hair, Delayed relaxation phase of the deep tendon reflexes

2.1.4.4.2.3 Diagnosis

The serum TSH concentration is elevated in primary hypothyroidism and its determination is an appropriate screening test for thyroid dysfunction. If the differential diagnosis includes central hypothyroidism or if the overall suspicion for overt hypothyroidism is high, fT4 should be included. In mild hypothyroidism, serum fT3 can remain in the normal range due to the increased conversion of fT4 to fT3 by D2 and the preferential secretion of fT3 by residual thyroid tissue under the influence of high TSH levels. [169] For these reasons, measurement of the serum T3 and fT3 concentration is not a useful test in the diagnosis or monitoring of patients with primary hypothyroidism. The presence of goiter or high TSH levels should prompt the measurement of TPOAb. TPOAb are the most sensitive screen for AT. Little further benefit is gained by the additional measurement of TgAb, although they may be added if TPOAb are negative. [170] Ultrasonography of the gland shows characteristic structural abnormalities such as generalized hypoechoicity and disomogeneity, due to inflammation and diffuse lymphocytic infiltration with occasional germinal centers (pseudonodules). A diffuse fibrosis of the gland can become evident at a later stage of the disease. [171]

The typical patient with hypothyroidism secondary to AT will have an elevated TSH ("typically" over 10 IU/mL), a low fT4, and positive TPOAb. In early stages of the disease, TSH may be normal and TPOAb may be positive with or without goiter. Later, TSH elevation becomes modest (5–10 IU/mL) with a normal fT4 (biochemical or subclinical hypothyroidism). Up to (90%) of patients with hypothyroidism secondary to AT are TPOAb positive. It should be noted that (10–15%) of the general population are positive for TPOAb and that low titers less than (1/100) by agglutination

methods or less than (100 IU/L) by immunoassays are less specific for ATDs. [136]

If TPOAb are absent, less common etiologies of primary hypothyroidism should be considered: transient hypothyroidism due to postsubacute thyroiditis, hypothyroidism related to external irradiation, [172] and consumptive hypothyroidism due to the inactivation of thyroid hormone by the paraneoplastic expression of D3, mostly in vascular tumors. [173] Subclinical hypothyroidism is defined as TSH elevation with normal concentrations of circulating thyroid hormones fT4 and fT3. The log-linear relationship between serum TSH and fT4 explains how small reductions in serum fT4 lead to large deviations in TSH. The majority of these patients are asymptomatic, but studies in the adult population suggest that individuals with the combined risk factors of TSH level above the normal limit and positive thyroid antibodies TgAb or TPOAb are at high risk for progression to overt hypothyroidism. For this reason, we recommend thyroid hormone replacement in all patients with TSH values (>10 IU/mL) or with TSH values (>5 IU/mL) in combination with goiter or thyroid autoantibodies. [174]

The Link between grave's disease and Auto immune thyroiditis

The observation that the autoimmune attack against the thyroid gland could result in two opposing clinical phenotypes, AT and GD has been intriguing for decades. In AT, the lymphocytic infiltration of the thyroid gland leads to apoptosis of thyroid cells and hypothyroidism. In contrast, in GD the lymphocytic infiltration of the thyroid leads to activation of TSHr-reactive B cells that secrete TSHr - stimulating antibodies causing hyperthyroidism. The etiology of AT and GD involves common pathways in which thyroid reactive T cells escape tolerance and infiltrate the thyroid, and unique pathways in which these thyroid-reactive (T cells) either cause thyroid cell

death in AT or stimulation in GD. Although GD and AT have different clinical phenotypes and the mechanisms leading to their dichotomy are unknown, they are generally believed to share a number of common etiological factors. There have been reports on monozygotic twins in whom one twin had GD and the other one had. [175, 176] Moreover, both conditions may aggregate in the same family [177] or may even coexist in the same thyroid gland, [178] and some individuals may progress from one form to the other. It is more frequent that GD may spontaneously culminate in hypothyroidism due to AT, [179] while the development of GD from AT as only occasionally been reported. [180, 181] On the other hand, whole genome scanning studies in humans have revealed differences between the specific loci linked to, or associated with, these two ATDs. [182]

2.1.4.4.3 Grave's disease (GD)

Robert Graves reported the clinical syndrome of goiter, palpitations, and exophthalmos in 1835. In adults, GD accounts for (60–80%) of all patients with hyperthyroidism, Hyperthyroidism is relatively rare in children (yearly incidence of (8 per 1,000,000) children less than (15 years) old and (1 per 1,000,000) children (< 4 years old), but GD is by far the most common etiology. Girls are affected four to five times more frequently than boys, although no gender difference is noted less than 4 years of age. [183]

2.1.4.4.3.1 Pathophysiology

GD shares many characteristics with AT, including TgAb, TPOAb, and antibodies against the sodium - iodine symporter. Hyperthyroidism is caused by thyroid-stimulating antibodies that bind and activate TSHr, leading to follicular cell hyperplasia and hypersecretion of thyroid hormones. Lymphocytic infiltration of the thyroid is present. Sometimes, germinal centers appear and develop as major sources of intrathyroid autoantibodies.

The lymphocytic infiltration and the accumulation of glycosaminoglycans in the orbital connective tissue and skin cause the extrathyroidal manifestations of GD ophthalmopathy and dermopathy, respectively. [184]

2.1.4.4.3.2 Clinical Aspects

The presentation of GD in childhood may be insidious and a careful history often reveals a several month history of progressive symptoms. Children may have the same signs and symptoms of hyperthyroidism as do adults, but most often they present with behavioral disturbances: decreased attention span, difficulty concentrating (which may lead to deteriorating performance in school), emotional lability, hyperactivity, difficulty sleeping, and nervousness. Typical cardiovascular findings include tachycardia, palpitations, widened pulse pressure, and an overactive precordium. Any child who has persistent tachycardia should be evaluated for hyperthyroidism. Tremors, a shortened deep tendon reflex relaxation phase, fatigue, and proximal muscle weakness are possible neuromuscular manifestations of thyrotoxicosis. Despite an increase in appetite, affected children often lose weight and sometimes have diarrhoea, but usually have frequent bowel movements associated with intestinal motility. Increased perspiration, warmth, and heat intolerance tend to be late findings. Postpubertal girls often have menstrual irregularities. A goiter is palpable in the majority of cases, characterized by diffuse enlargement which is smooth, firm, and nontender. The pretibial myxedema that is a common feature of **GD** in adults is rare in children. [184]

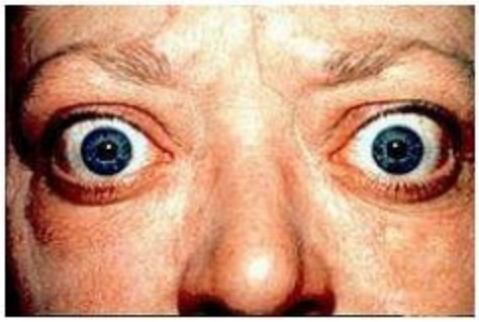

Figure (2.6) Exophthalmoses [Medicinenet.com]

Clinical signs and symptoms of hyperthyroidism in children

Goiter, Exophthalmos, Acceleration of linear growth, Irritability, Impaired concentration and school performance, Headache, Hyperactivity, Fatigue, Palpitations, Tachycardia, Systolic Hypertension, Polyphagia, Increased frequency of bowel movements with diarrhoea, Weight loss, Heat intolerance, Increased perspiration, Tremor, Polyuria and polydipsia.

Extrathyroidal manifestations such as ophthalmopathy and dermopathy are rarer in children than in adults and tend to be less severe. [184] A (25–60%) frequency of ocular manifestations has been estimated in children, but usually the ocular signs are mild such as lid retraction, a slight proptosis that can be attributed to the inflammation and muscle swelling rather than to infiltrative disease of the orbital structures. As expected, these signs improve in most patients after restoration of the euthyroid state. [184] Unique to pediatric GD is the acceleration of linear growth and bone maturation associated with prolonged hyperthyroidism. [185, 186]

2.1.4.4.3.3 Diagnosis

Even if there may be national differences in terminology, for the purposes of this study the term thyrotoxicosis refers to the manifestations of excessive quantities of circulating thyroid hormones. On the contrary, hyperthyroidism refers only to the group of diseases which are due to the overproduction of

hormones by the thyroid gland. An accurate diagnosis of GD is critical as antithyroid drugs have no role in the treatment of thyrotoxicosis without hyperthyroidism. Thyrotoxicosis is recognized by an elevation of serum fT4 with a decreased serum TSH (typically 0.1μIU/mL). A determination of the fT3 level should be added if TSH is suppressed and the serum fT4 is normal. In patients with early disease or in iodine-deficient patients, serum fT4 concentrations may be normal or reduced despite elevated levels of fT3. Once biochemical derangement has been documented, it is helpful to address the duration of thyrotoxicosis to facilitate the differentiation of GD from other causes of thyrotoxicosis. Onset may be documented by prior laboratory studies or inferred from the history. The differential diagnosis of thyrotoxicosis includes transient thyroiditis, hyperfunctioning nodules, and thyrotoxicosis factitia. In the majority of cases, the presence of a symmetrically enlarged thyroid gland, coupled with the chronicity of symptoms, will be adequate to allow a diagnosis. If thyrotoxicosis has been present for more than (8 weeks), GD is by far the most likely etiology. The constellation of thyrotoxicosis, goiter, and orbitopathy is pathognomonic of this condition and no additional laboratory tests or imaging studies should be necessary to confirm the diagnosis. If thyromegaly is subtle and eye changes are absent, a thyroid echography should be performed. The radioactive iodide uptake RAIU should be reserved for patients in whom a discrete nodule(s) is palpable or evident at ultrasonography. In patients with a toxic nodule, iodide uptake will localize to the nodule and the signal in the surrounding tissue will be low, secondary to TSH suppression. Thyrotoxicosis factitia can be recognized by a low RAIU and serum Tg, in the presence of thyrotoxicosis and suppressed TSH levels. If thyrotoxicosis has been present for less than 8 weeks, transient thyrotoxicosis secondary to

subacute thyroiditis or the thyrotoxic phase of AT should be considered. An elevated sedimentation rate supports subacute thyroiditis whereas increased TPO and Tg without increased TSHr antibody titers supports the latter. RAIU was used in the past decades to distinguish thyrotoxicosis due to the different forms of thyroiditis (increased release of thyroid hormone low RAIU, from the more common GD (increased production of thyroid hormone high RAIU, but the measurement of TSHr antibodies may now offer an effective tool to make the correct diagnosis, and RAIU is no more indicated for differential diagnosis. TSHrAb are commonly present in GD, whereas they are absent from AT and in the other forms of thyrotoxicosis. The sensitivity of two frequently used serum TSHrAb assays is cited to be (75–96%) for TBII a competitive binding assay with TSH and (85–100%) for TSAb measurements a bioassay of TSHr activation in untreated GD patients. A false negative rate of (10–20%) has been documented for serum TSHrAb in GD, presumably due to the inadequate sensitivity of the assays, or the exclusive intrathyroidal production of autoantibodies. [68]

Differential diagnosis of thyrotoxicosis in children

- Thyrotoxicosis associated with sustained hormone overproduction.
- High RAIU.
- Graves' disease.
- Toxic multinodular goiter.
- Toxic adenoma.
- Increased TSH secretion (TSH secreting adenomas).
- Thyrotoxicosis without associated hyperthyroidism Low RAIU.
- Thyrotoxicosis factitia.
- Subacute thyroiditis.
- Chronic autoimmune thyroiditis.

- Ectopic thyroid tissue (struma ovarii, functioning metastasis of differentiated thyroid cancer).

In practice, the measurement of TSHrAb is routinely used in children to avoid RAIU, as the combination of clinical signs, symptoms of thyrotoxicosis, and positive autoantibodies, in the absence of a nodule at ultrasonography, is virtually diagnostic of GD. There is a subgroup of patients who have a subnormal but not severely depressed TSH usually (0.1–0.3μIU/mL) and normal serum concentrations of thyroid hormones. These patients are generally asymptomatic and the term "subclinical hyperthyroidism" has been applied to their condition. In elderly people, a low serum TSH concentration has been associated with an increased risk of atrial fibrillation, but no similar risks have been identified in the paediatric population.[68] Furthermore, several studies indicate that approximately half of patients with subclinical thyrotoxicosis will experience a spontaneous remission. [129] The initial detection of a suppressed TSH concentration, without elevated levels of thyroid hormone or associated symptoms, should be addressed simply by repeating thyroid function tests in (4–8 weeks). Assuming there are no specific risk factors such as a history of cardiac disease, asymptomatic children with subclinical hyperthyroidism can be followed with the expectation that TSH suppression due to transient thyroiditis will resolve spontaneously and that due to GD or autonomous secretion will declare itself clinically over time. [129]

2.1.4.4.4 Neonatal Grave's Disease

Thyroid hormones are necessary for optimal fetal and neonatal development, and the risk of malformations may be increased in the newborns to hyperthyroid mothers. [187, 188] Lack of thyroid hormones for more than a few weeks, during vulnerable periods of development, involves a risk of

permanent cerebral impairment. [189] Conversely, excess amounts of thyroid hormone are associated with increased risk of fetal death and may lead to accelerated bone maturation leading to early epiphyseal fusion and growth cessation. Also long-term exposure may lead to ostepenia in adolescence and adulthood. [190] Only (0.6%) of infants born to mothers with a history of GD will develop neonatal hyperthyroidism, due to the transplacental passage of thyroid-stimulating immunoglobulins. Even after definitive treatment by I^{-131} or thyroidectomy, women with a history of ATDs are at risk for fetal and neonatal thyroid dysfunction secondary to the persistence of maternal autoantibodies. The pregnancy of such women should be considered high risk, and the care should be coordinated between an experienced obstetrician and an endocrinologist. Fetal heart rate and growth should be monitored by regular prenatal ultrasounds. The measurement of TSHrAb during at-risk pregnancies has been recommended as a predictor for the development of fetal/neonatal GD. [191] Highly experienced ultrasonographers can often visualize the fetal thyroid. The presence of foetal goiter, tachycardia, and intrauterine growth retardation suggests foetal hyperthyroidism. In these rare patients, antithyroid drugs are administered to the mother to control fetal hyperthyroidism; this will keep the fetus euthyroid until birth. After birth, the antithyroid drugs from the mother will disappear from the fetal circulation within the first days of life. After some delay, neonatal hyperthyroidism may develop and remain until the maternal antibodies are cleared. Pediatricians should be aware that the use of maternal antithyroid medications near the time of delivery or the co-transfer of maternal anti-TSHr blocking immunoglobulins may delay the appearance of neonatal GD. [190] For high-risk infants, such as those born to mothers with high levels of TSHrAb stimulating antibodies or those with a history of an affected sibling,

clinical monitoring and thyroid function tests at birth and at (1 and 2 months) of age are recommended. [192] An additional set of laboratory tests at (1 week) of age is indicated for infants who have been exposed to maternal antithyroid drugs in the third trimester. Affected infants are often flushed, diaphoretic, and hyperkinetic. Goiter is common and, when severe, can endanger the infant's airway. Diarrhoea, vomiting, poor weight gain, and a transient exophthalmos may be seen. Arrhythmias and/or congestive heart failure can develop and require treatment with digoxin. Serum for confirmatory thyroid function tests TSH, fT4 should be obtained and treatment initiated immediately. [192]

Almost one-third of the world's population lives in areas of iodine deficiency. [190] In areas where the daily iodine intake is 50µg, goiter is usually endemic, and when the daily intake falls, (25µg), congenital hypothyroidism is seen. The prevalence of goiter in areas of severe iodine deficiency can be as high as (80%). Populations at particular risk tend to be remote and live in mountainous areas in South-East Asia, Latin America and Central Africa. Iodization programmes are of proven value in reducing goiter size and in preventing goiter development and cretinism in children. Autonomy can develop in nodular goiters leading occasionally to thyrotoxicosis and iodization programme can also induce thyrotoxicosis, especially in those aged (40 years) with nodular goitres. [193]

In iodine-replete areas, most persons with thyroid disorders have autoimmune disease, ranging from primary atrophic hypothyroidism, HT to thyrotoxicosis caused by GD. Cross-sectional studies in Europe, the (USA) and Japan have determined the prevalence of hyperthyroidism and hypothyroidism and the frequency and distribution of thyroid autoantibodies in different, mainly Caucasian, communities. [193] Data from screening large

US population Samples [194, 195] have revealed differences in the frequency of thyroid dysfunction and serum thyroid antibody concentrations in different ethnic groups, whereas studies from Europe have revealed the influence of dietary iodine intake on the epidemiology of thyroid dysfunction.[196] Studies of incidence of autoimmune thyroid disease have only been conducted in a small number of developed countries. [197]

2.1.4.4.5 Congenital hypothyroidism

Congenital hypothyroidism affects about one newborn in (3500–4000) birth and is the most treatable cause of mental retardation. [198] There is an inverse relationship between age at diagnosis and intelligence quotient in later life. In iodine-replete areas, (85%) of the cases are due to sporadic developmental defects of the thyroid gland (thyroid dysgenesis), such as the arrested migration of the embryonic thyroid (ectopic thyroid) or a complete absence of thyroid tissue (athyreosis). The remaining (15%) have thyroid dyshormonogenesis defects transmitted by an autosomal recessive mode of inheritance. A daily iodine intake (<25µg), particularly in preterm infants, accounts for many cases in Europe, Asia and Africa. Clinical diagnosis occurs in (<5%) of newborns with hypothyroidism because symptoms and signs are often minimal. As a result, it is not possible to predict which infants are likely to be affected. Without prompt diagnosis and treatment most affected children gradually develop growth failure, irreversible mental retardation and a variety of neuropsychological deficits. [198]

Thyroid hormone assays

Only very small fractions of thyroid hormones are not bound to protein. These free thyroid hormones are the physiologically important thyroid hormones in blood. Modern immunoassays that estimate free hormone concentrations are widely available. Changes in serum albumin

concentrations, abnormal binding proteins, free fatty acids and drugs such as heparin, frusemide and phenytoin may interfere with these assays. Most laboratories now use chemiluminescent methods that are more (but not completely) resistant to such interference. When results do not fit into a recognized pattern the laboratory should be consulted to identify such interferences. [194]

2.1.5 Thyroid-related autoantibodies

If a person has altered thyroid function, testing for thyroid antibodies helps to determine if they have an autoimmune condition. [200, 201]

2.1.5.1 Thyroperoxidase autoantibodies

TPOAbs are also known as thyroid microsomal antibodies. They are present in autoimmune thyroid disease, but there is debate about whether low levels are always pathological. Unfortunately, there are significant differences between laboratories when the same sera are studied, and lower detection limits are variable. Assay sensitivities and reference ranges can therefore vary quite widely.

TPOAbs can cause hypothyroidism in at least two ways. Firstly they can block TPO thereby inhibiting T4 and T3 synthesis and secondly through antibody-dependent cell cytotoxicity and thyroid inflammation. Low concentrations may not be associated with evidence of thyroid dysfunction, but the incidence of raised TSH increases as antibody levels rise. The prevalence of positive antibody levels and mild hypothyroidism increases with age. The concentration of TPOAbs may fluctuate in patients with autoimmune thyroid disease. This has no clinical significance and repeated measurements are not recommended. Maternal TPOAbs cross the placenta, but their effects on fetal thyroid function are unclear. [200, 202]

2.1.5.2 Thyroglobulin autoantibodies

TgAbs are also a marker of autoimmune thyroid disease, but are less common than TPOAb. TgAb do not inhibit TPO or mediate antibody-dependent cell cytotoxicity and are therefore markers rather than mediators of autoimmune thyroid disease. There are considerable variations in sensitivity and reference ranges between assays. Other autoimmune diseases can also increase the concentration of TgAb. [200, 202]

2.1.5.3 TSH receptor autoantibodies

TSHrAb may stimulate or less commonly block the TSHr. Stimulating antibodies cause GD and probably also cause the associated ophthalmopathy. Blocking antibodies can cause hypothyroidism. The assay of TSHrAb done in clinical laboratories cannot distinguish between stimulating and blocking antibodies. This is not usually relevant as clinical hyperthyroidism would suggest that the dominant antibody is stimulatory. Measuring TSHrAb can be useful if the cause of hyperthyroidism is not apparent. However, initial hopes that remission of GD could be predicted by falling autoantibody levels have not been supported by most studies. Measurements of TSHrAb do have an important role in managing pregnant women with GD. High concentrations of maternal TSHrAb can predict fetal and neonatal hyperthyroidism. It is important to recognize that TSHrAb do not always fall after successful treatment, so pregnant women with a previous history of GD should be screened for TSHrAb. [200, 202]

2.1.5.4 Thyroglobulin

Tg, a large glycoprotein, represents about (80%) of the wet weight of the thyroid and is co-secreted with thyroid hormone. Concentrations are high in patients with raised TSH concentrations or nodular goiters, but it is not clinically useful to measure Tg in these situations. Most papillary and

follicular carcinomas synthesize and secrete thyroglobulin, but raised Tg levels are not a reliable indicator or screening test for thyroid malignancy. Tg concentration becomes a useful marker of remaining or recurrent cancer in patients who have had a total thyroidectomy and remnant ablation with radioiodine for papillary and follicular carcinoma. Unfortunately, up to (20%) of patients with differentiated thyroid cancer have TgAb that interfere with the thyroglobulin assay, leading to underestimation of Tg concentration. TgAb should therefore be measured, with a sensitive assay, on all Tg samples. [200, 202]

Screening for thyroid disorders

Thyroid nodules may be detected because of their size or anterior position in the neck, or the skill of the physician performing the examination. However, most thyroid nodules are not clinically recognized. Ultrasonography as a screening tool is too sensitive and will result in unnecessary pursuit of findings, which are so common that they rarely have pathological significance. However, it may have a place in investigating patients presenting with thyroid nodules to determine whether they are single or multiple. As diagnostic techniques for thyroid cancer have become more sensitive, particularly with the advent of ultrasound and fine-needle aspiration, there has been an increased detection of subclinical papillary cancers. Epidemiological data suggest that the children of women with hypothyroxinemia may have psychoneurological deficits. [203] In classic areas of iodine deficiency, a similar range of deficits in children has been described where maternal hypothyroxinemia rather than high- serum TSH is the main biochemical abnormality. In these areas, maternal iodine intake is often substantially (<200mg per day) currently recommended. Even in areas previously thought to be iodine sufficient, there is now evidence of

substantial gestational iodine deficiency, which may lead to low maternal circulating T4 concentrations. In addition to the childhood neuropsychological problems relating to low T4 values, there is evidence that maternal TPOAb may result in intellectual impairment even when there is normal thyroid function. [203] The value of screening for congenital hypothyroidism in heel-prick blood specimens is unquestioned, and it is now done routinely in many countries. Controversy exists as to whether healthy adults living in an area of iodine sufficiency benefit from screening for thyroid disease. The benefit from a screening programme must outweigh the physical and psychological harm caused by the test, diagnostic procedures and treatment. [204] The prevalence of unsuspected overt thyroid disease is low, but a substantial proportion of subjects tested will have evidence of thyroid dysfunction, with (~10%) with subclinical hypothyroidism and (1%) with subclinical hyperthyroidism. No appropriately powered prospective, randomized, controlled, double-blinded interventional trial of either levothyroxine therapy for subclinical hypothyroidism or anti-thyroid therapy for subclinical hyperthyroidism exists. [205]

In subclinical hypothyroidism, there is still debate as to what constitutes a normal serum TSH, particularly in older subjects. Although some subjects will progress to overt hypothyroidism, recent data suggest a significant proportion revert to normal without treatment. Recent meta-analyses have suggested increased cardiovascular risk in younger adults and in those with a serum (TSH >10 mIU/l). [208] Other data suggest that mild thyroid failure may be the only reversible cause of left ventricular diastolic dysfunction. [199] Treatment in those who are symptomatic, pregnant or pre-conception, aged (≥ 65 yrs) or evidence of heart failure appears justified. [206]

No consensus exists regarding the treatment of subclinical hyperthyroidism, although it has been strongly argued without any evidence-base that therapy with anti-thyroid drugs or radioiodine may be indicated in view of the long-term risk of atrial fibrillation and loss of bone density. Any potential benefits of therapy in subclinical hyperthyroidism must be weighed against the substantial morbidity associated with the treatment of thyrotoxicosis. For the vast majority of patients adopting a 'wait and see' policy rather than intervention may avoid unnecessary treatment or the potential for harm. [207]

2.2 Previous studies

Study conducted in Japan [209] revealed that**:** Serum levels of TgAb and TPOAb were measured by radioimmunoassay. Out of the (146) patients, (18) had detectable serum TgAb and (16) had detectable serum TPOAb. All but one (i.e. 94%) of the (18) TgAb positive patients had FLT and (14) out of the (16) TPOAb positive patients had FLT). [209]

Study conducted in South India [210] stated that**:** TPOAb tested positive in (89%) of patients and negative in (11%). TgAb estimation was positive in (64 %) of patients and negative in (36%). By thyroid function testing and serum antibody evaluation, of the (89) TPOAb positive patients, (60.7%) were hypothyroid, (6.7%) hyperthyroid, and (32.6%) euthyroid. Among euthyroid patients, (90%) were TPOAb negative. In (64) TgAb positive patients, (53.1%) patients were hypothyroid, (4.7%) hyperthyroid and (42.2%) euthyroid. But in the 36 TgAb negative patients, (58.3%) were hypothyroid. At the time of the first clinic visit, (55%) of patients were hypothyroid, (6%) hyperthyroid and (39%) euthyroid. Conclusion: In our study, TPOAb was more sensitive than TgAb in predicting hypothyroidism. Similarly, TPOAb was more sensitive than TgAb in autoimmune thyroiditis (98.1% vs. 61.8%, p value < 0.005). Hypothyroidism was the most frequent

thyroid dysfunction in patients with positive TPO and Tg antibodies. The absence of TPO usually is associated with no thyroid dysfunction, but the same cannot be said of Tg. [210]

Study done in Gujarat in India [211] to evaluate the variations in thyroid hormones in different age, gender, and seasons; they concluded that the age, gender and seasons have an appreciable effects on the levels T3, T4 and TSH Levels of T3, T4 and TSH ranged from (0.98-4.8ng/dl), (0.56-3-25ng/dl) and (0.01-5.3μIU/L). There is significant change in thyroid hormone levels in both genders of different age group in different seasons. [211]

Other study conducted in Iran [212] concluded that; among (91) type 1 diabetic patients, 36 (39.6%) were positive for TPOAb and 27(30%) were positive for anti TG. Anti-TPO antibodies were detected only in (6.7%) of control group. Comparing with those without thyroid autoimmunity, there was a female preponderance for the type 1 diabetic patients with thyroid autoimmunity (female: male, 28:14 vs. 28:20 respectively). Among the type 1 diabetic patients those with thyroid autoimmunity, tended to be older (p: 0.04) and to have higher TSH concentration p: 0.03. Patients with high TPOAb levels had longer duration of diabetes (P: 0.02). The presence of TPOAb in (39.6%) of type 1 diabetic patients comparing with (8.5%) of normal subjects confirmed the strong association of ATD and type 1 diabetes mellitus. [212]

Study conducted in Iran [213] to compare the prevalence of positive autoantibodies in patients with thyroid disorders and healthy subjects in an iodine-replete area of the Islamic Republic of Iran, it studied 930 women in a clinic-based study: 698 patients (286 hypothyroid, (140) hyperthyroid, (272) with simple goiter) and (232) healthy women. Serum T4, T3, TSH, and antithyroid antibodies were measured. Positive autoantibodies were

detected in (75.5%) of patients with hypothyroidism, (73.6%) of those with hyperthyroidism, (48.9%) of those with simple goiter and (35.8%) of the control group (P<0.001). Autoimmunity may have a role in the genesis of common thyroid disorders. [213]

Study conducted in Germany [214] to determine thyroid function tests in patients taking thyroid medication; they found that; TSH levels of (< 0.27 or > 2.15 mIU/L) in subjects younger than (50 years) and (< 0.19 or > 2.09 mIU/L) in subjects (50 years) and older, were defined as decreased or elevated, according to the established reference range for the specific study area. Analysis revealed that 56 of 190 (29.5%) subjects treated with thyroxine had TSH levels outside the reference range (10.0% elevated, 19.5% decreased). Of the (31) subjects taking antithyroid drugs, 12 (38.7%) had TSH levels outside the reference range (9.7% elevated, 29.0% decreased). These proportions were lower in the (45) subjects receiving iodine supplementation (2.2% elevated, 8.9% decreased). Among the (3,974) SHIP participants not taking thyroid medication, TSH levels outside the reference range (2.8% elevated, 5.9% decreased) were less frequent. [214]

In the study conducted [215] concluded that: Thyroid peroxidase TPO represents the major autoantigen in AIT. On this basis there is a moderate positive correlation between levels of TPOAb and risk of hypothyroidism. TPOAb in AIT should only be measured in the context of elevated serum TSH levels. [215]

Other study conducted in China [216] to evaluate the thyroid hormones on thyroid function, they conclude that; The correlation of TT4, and fT4 with TSH was statistically significant in healthy individuals (P < 0.01), and the R-values were (−0.065 and −0.152), respectively. The correlation of TT4, fT4, TT3, and fT3 with TSH was statistically significant in patients with

hyperthyroidism. The correlation of TT4, fT4, TT3, and fT3 with TSH was statistically significant in patients with hypothyroidism. In our opinion, TSH and fT4 are the most valuable indicators in assessing thyroid function in a healthy population, and TSH and TT4 are the most meaningful in hyperthyroidism and hypothyroidism. [216]

Other study conducted in **USA** [217] and revealed that the prevalence of the TPOAb in the high-normal group was (18.6%) versus (3%) in the low-normal range TSH. The TPOAb prevalence was higher in females than in males and had a racial predominance in Hispanics compared to African Americans; however, these differences were not statistically significant. They conclude that: TPOAb measurement may be appropriate for patients with high-normal TSH to help distinguish those at risk of developing true hypothyroidism. [217]

CHAPTER THREE

Materials and methods

3. Materials and Methods

3.1. Study design

This was prospective, case - control, hospital based study carried out from 2013-2017 in Shendi locality

3.2. Study area

Shendi locality (River Nile State-Sudan) is located north of Khartoum, about (176 km). The total area of the Shendi locality is about (1496 km^2). Shendi locality population about 269446 males (48.7%), females (51.3%) according to 2008 consensus, most of the people are farmers.

3.3. Study population

The study included the following

• Thyroid diseases patients in Shendi locality.

• The thyroid diseases patient's criteria are:

Patients who visit Elmek Nemir hospital outpatient clinic to routine follow up, clinics of physicians, during the time of the study

The control group criteria

Healthy subjects without thyroid diseases and match with study group in age and sex distribution.

3.4. Sampling

3.4.1. Sampling technique

Random sampling was used to select suitable sample size.

3.4.2. Sample size

283 participants from the population of this study were divided into three groups:

- Group one: control group (healthy) 100 subjects.
- Group two: hyperthyroidism patients.

- Group three: hypothyroidism patients.

3.5. Data collection

Structured questionnaire was used to collect the following data; personal data, social customs, food habits, exercise, medical history, weight, height, duration of the disease, type of thyroid drugs.

3.5.1. Sample collection

Venous blood samples (3ml) were drawn in heparinized blood collection tubes, using sterile syringes and centrifuged (1500 r.p.m) for five minutes to obtain heparinized plasma for analysis of thyroid hormones profile (TSH, T4, T3, fT3, and fT4) Samples were obtained from the thyroid disease patients and healthy group as control.

Aliquots of (2) ml were collected in plain container, and were allowed to clot and then centrifuged (1500 r.p.m) for five minutes, the supernatant sera were transferred into a plastic tube (eppindorff tube) and stored at (-80^{o}C) for the analysis of thyroid antibodies (antithyroid peroxidase, antithyroglobulin antibodies)

3.6 Methodology

3.6.1 Thyroid stimulating hormone measurement

ST AIA-PACK TSH

For quantitative measurement of thyroid stimulating hormone in serum or heparinized plasma

Principle of the assay

The ST AIA – PACK TSH is a two – site immunoenzymometric assay which is performed entirely in the ST AIA – PACK TSH test cups. TSH present in the test sample is bound with monoclonal antibody immobilized on magnetic beads and monoclonal antibody conjugated with bovine alkaline phosphatase in the test cups. The magnetic beads are washed to

remove unbound enzyme – labeled monoclonal antibody and are then incubated with a Fluorogenic substrate. 4 – Methylumbelliferyl phosphate (4MUP). The amount of enzyme conjugated with monoclonal antibody that binds to the beads is directly proportional to the TSH concentration in the sample. A standard curve is constructed, and unknown sample concentrations are calculated using this curve.

Material provided (ST AIA – PACK TSH, Cat. No. 0025294)

5 trays * 20 test cups

Plastic test cups containing lyophilized twelve magnetic beads coated with anti – TSH mouse monoclonal antibody and (50 µL) of anti – TSH mouse monoclonal antibody conjugated to bovine alkaline phosphatase with sodium azide as a preservative.

Procedure

Calculation of results

The TOSOH AIA system analyzer performs all sample and reagent handling operations automatically. The TOSOH AIA system analyzers read the rate of fluorescence produced by the reaction and automatically convert the rate to TSH concentration in (µIU/mL).

3.6.2 Thyroxine TT4 measurement (ST AIA – PACK T4)

For quantitative measurement of TT4 in serum or heparinized plasma

Principle of the assay

The ST AIA – PACK T4 is a competitive enzyme immunoassay which is performed entirely in the ST AIA – PACK T4 test cups. Thyroxine, which is displaced from its binding proteins by ANS (8 – anilino – 1 - naphthalene sulfonic acid) and fT4 present in the test sample complete with enzyme – labeled thyroxine for a limited number of binding sites on a thyroxine – specific antibody immobilized on magnetic beads. The beads are washed to

remove unbound enzyme – labeled thyroxine and are then incubated with a Fluorogenic substrate. (4MUP), the amount of enzyme – labeled that binds to the beads is inversely proportional to the thyroxine concentration in the sample. A standard curve using a range of known standard concentration is constructed and unknown thyroxine concentrations are calculated using this curve.

Materials provided (ST AIA – PACK T4, Cat. No 0025258)

5 trays * 20 test cups

Plastic test cups containing lyophilized twelve magnetic beads with anti – T4 rabbit polyclonal antibody, (140 µL) of T4 conjugated to bovine alkaline phosphatase and ANS with sodium azide as a preservative.

3.6.3 Free thyroxine FT4 measurement (ST AIA – PACK FT4)

For quantitative measurement of non – protein bound (free) thyroxine (fT4) in serum or heparinized plasma

Principle of the assay

The ST AIA – PACK FT4 is a competitive enzyme immunoassay which is performed entirely in the ST AIA – PACK FT4 test cups. The thyroxine not bound to serum proteins (free T4) competes with enzyme – labeled T4 for limited number of binding sites on a T4 – specific antibody immobilized on magnetic beads. After incubation, the beads are washed to remove the unbound enzyme – labeled T4 and are then incubated with a Fluorogenic substrate, (4MUP). The amount of enzyme – labeled T4 that binds to the beads is inversely proportional to the free T4 concentration in the test sample. A standard curve using a range of known standard concentrations is constructed and unknown sample free T4 concentrations are calculated using this curve.

Materials provided (ST AIA – PACK FT4, Cat. No 0025268)

5 trays * 20 test cups

Plastic test cups containing lyophilized twelve magnetic beads with anti – T4 rabbit polyclonal antibody, 140 µL of thyroxine T4 conjugated to bovine alkaline phosphatase with sodium azide as a preservative.

3.6.4 Triiodothyronine TT3 measurement (ST AIA – PACK TT3)

For quantitative measurement of total triiodothyronine (TT3) in serum or heparinized plasma

Principle of the assay

The ST AIA – PACK TT3 is a competitive enzyme immunoassay which is performed entirely in the ST AIA – PACK TT3 test cups. Triiodothyronine, which is displaced from its binding proteins by ANS, and free T3 present in the test sample compete with enzyme – labeled T3 for a limited number of binding sites on a T3 specific antibody immobilized on magnetic beads. The beads are washed to remove the unbound enzyme – labeled T3 and are then incubated with a Fluorogenic substrate, (4-MUP). The amount of enzyme – labeled T3 that binds to the beads is inversely proportional to the T3 concentration in the test sample. A standard curve using a range of known standard concentrations is prepared and unknown T3 concentrations are calculated using this curve.

Materials provided (ST AIA – PACK TT3, Cat. No 0025282)

5 trays * 20 test cups

Plastic test cups containing lyophilized twelve magnetic beads with anti – T3 sheep monoclonal antibody, 125µL of T3 conjugated to bovine alkaline phosphatase and ANS with sodium azide as a preservative.

3.6.5 Free triiodothyronine FT3 measurement (ST AIA – PACK iFT3)

For quantitative measurement of free triiodothyronine (FT3) in serum or heparinized plasma

Principle of the assay

The ST AIA – PACK iFT3 is a competitive enzyme immunoassay which is performed entirely in the ST AIA – PACK iFT3 test cups. Free triiodothyronine (FT3) present in the test sample compete with enzyme – labeled triiodothyronine (T3) for a limited number of binding sited on a T3 – specific antibody immobilized on the magnetic beads. The beads are washed to remove the unbound enzyme – labeled free triiodothyronine and are then incubated with a Fluorogenic substrate, 4-MUP. The amount of enzyme – labeled free triiodothyronine that binds to the beads is inversely proportional to the free triiodothyronine concentration in the sample. A standard curve using a range of known standard concentrations is constructed and unknown free triiodothyronine concentrations are calculated using this curve.

Materials provided (ST AIA – PACK iFT3, Cat. No 0025231)

5 trays * 20 test cups

Plastic test cups containing lyophilized twelve magnetic beads with anti – T3 rabbit monoclonal antibody, and 50 µL of T3 conjugated to bovine alkaline phosphatase with sodium azide as a preservative.

3.6.6 Anti-thyroid peroxidase measurement (Anti-TPO) ELISA

Principle of the test: a sequential Elisa method

The reagents required for the sequential ELISA assay include immobilizes antigen, circulating autoantibody and enzyme-linked species –specific antibody. In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of Streptavidin coated on the well and exogenously added biotinylated thyroid peroxidase

antigen. Upon mixing biotinylated antibody and a serum containing the autoantibody, a reaction results between the antigen and the antibody to form an immune complex.

The interaction is illustrated by the following equation:

$$^{\text{h-Ab}}(\text{x-TPO}) + {}^{\text{Bin Ag}}(\text{TPO}) \underset{\text{K}-\text{a}}{\overset{\text{Ka}}{\rightleftharpoons}} {}^{\text{h-Ab}}(\text{x-TPO}) - {}^{\text{Bin Ag}}(\text{TPO})$$

Bin Ag (TOP) = biotinylated antigen (constant quantity)

h-Ab (x-TPO) = human auto-antibody (variable quantity)

Ab(x-TPO) – BinAg (TPO) = immune complex (variable quantity)

Ka = rate constant of association

K-a = rate constant of disassociation

Simultaneously, the complex is deposited to the well through the high affinity reaction of Streptavidin and biotinylated antibody.

After a suitable incubation period, the well is washed to separate the unbound components by aspiration and/or decantation. The enzyme linked species-specific antibody (anti- h- IgG) is then added to the microwells. This conjugates binds to the immune complex that formed.

The anti-h-IgG enzyme conjugate that binds to the immune complex in a second incubation is separated from unreacted material by a wash step. The enzyme activity in this fraction is directly proportional to the antibody concentration in the specimen. By utilizing several different serum references of known antigen concentration, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained. [(218)]

3.6.7 Anti-thyroglobulin measurement (Anti-Tg) ELISA

Principle of the test: a sequential Elisa method:

The reagents required for the sequential ELISA assay include immobilizes antigen, circulating autoantibody and enzyme-linked species –specific antibody. In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of Streptavidin coated on the well and exogenously added biotinylated thyroglobulin antigen. Upon mixing biotinylated antibody and a serum containing the autoantibody, a reaction results between the antigen and the antibody to form an immune complex.

The interaction is illustrated by the following equation:

$$^{\text{h-Ab}}(\text{x-Tg}) + {}^{\text{Bin Ag}}(\text{Tg}) \frac{\text{Ka}}{\text{K}-\text{a}} \; {}^{\text{h-Ab}}(\text{x-Tg}) - {}^{\text{Bin Ag}}(\text{Tg})$$

Bin Ag (Tg) = biotinylated antigen (constant quantity)

h-Ab (x-Tg) = human auto-antibody (variable quantity)

Ab(x-Tg) – BinAg (Tg) = immune complex (variable quantity)

Ka = rate constant of association

K-a = rate constant of disassociation

Simultaneously, the complex is deposited to the well through the high affinity reaction of Streptavidin and biotinylated antibody.

After a suitable incubation period, the well is washed to separate the unbound components by aspiration and/or decantation. The enzyme linked species-specific antibody (anti- h- IgG) is then added to the microwells. This conjugates binds to the immune complex that formed.

The anti-h-IgG enzyme conjugate that binds to the immune complex in a second incubation is separated from unreacted material by a wash step. The enzyme activity in this fraction is directly proportional to the antibody

concentration in the specimen. By utilizing several different serum references of known antigen concentration, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained. [218]

3.7. Ethical considerations

The study was approved by ethical committee of the College of Graduate Studies &Scientific Research (Institute Research Board) of the Shendi University, before conducting the study permission was taken from Elmak Nimir Hospital general manager. Then verbal permission was taken from all participants after explaining the research aims and benefits, all of them agreed to participate, and they have the right to refuse any time during the study.

3.8. Data analysis

The collected data was analyzed by computer, using the statistical programs software Statistical Package for the Social Sciences (SPSS) version (11.5).

The following statistical measures were used:-

- Mean, Standard SD, frequency, percentage for quantitative data.
- T-test and correlation were used for qualitative data (significance level were set at $P \leq 0.05$)
- The data was presented in form of figures and tables.

CHAPTER FOUR

Results

4. Results

This study was conducted in Shendi locality to determine the sensitive thyroid parameters and thyroid antibodies in noncancerous thyroid disease patients, in the period of (2014-2017). The study included (183) patients divided into two groups, group (1) hyperthyroidism patients, group (2) hypothyroidism patients and compared with (100) healthy volunteers as a control group.

Table (4.1): Sex distribution among the study group

Sex groups	N=111		N=72	
	Hypothyroidism		Hyperthyroidism	
	Frequency	Percentage %	Frequency	Percentage %
Male	9	8.1	11	15.3
Female	102	91.9	61	84.7

Table (4.1): showed that in **hypothyroidism**; (91.9%) were female and (8.1%) were male, and in **hyperthyroidism**; (84.7%) were female and (15.3%) were male

Table (4.2): Family history among the study group

Family history	N=111		N=72	
	Hypothyroidism		Hyperthyroidism	
	Frequency	Percentage %	Frequency	Percentage %
Yes	37	33.3	27	37.5
First degree	24	64.9	19	70.4
Second degree	13	11.7	8	29.6
No	74	66.7	45	62.5

Table (4.2): denoted family history distribution; in **hypothyroidism**; (33.3%) were with family history, (64.9%) of them with first degree and (35.1%) were with second degree of family history, (66.7%) without family history. And in **hyperthyroidism**; (37.5%) were with family history (70.4%) of them with first degree and (29.6%) with second degree and (62.5%) without family history.

Table (4.3): Age and weight among the study group

Study group	N = 111			N = 72		
	Hypothyroidism			Hyperthyroidism		
	Frequency	Mean	SD	Frequency	Mean	SD
Age	111	50.4	14.7	72	43.6	13.4
Weight	111	68.1	15.5	72	64.9	13.5

Table (4.3): indicated the distribution of study group according to age and weight; in **hypothyroidism**; the mean of age 50.4± 14.7 years, in hyperthyroidism was 43.6±13.4 years, and the weight in hypothyroidism was 68.1±15.5Kgm, and in **hyperthyroidism** was 64.9±13.5kg.

Table (4.4): Discovery of cases among the study group

	N= 111		N = 72	
	Hypothyroidism		Hyperthyroidism	
	Frequency	Percentage %	Frequency	Percentage %
New cases	56	50.5	30	41.7
Under treatment	55	49.5	42	58.3

Table (4.4): revealed the distribution of study group according to discovery of cases; in **hypothyroidism**, (50.5%) were new discovered cases and (49.5%) were under treatment, and in **hyperthyroidism**; (41.7%) were new cases and (58.3%) under treatment.

Table (4.5): Thyroid peroxidase antibodies among test group

TPOAb results	N= 111		N = 72		Total	
	Hypothyroidism		Hyperthyroidism			
	Frequency	Percentage %	Frequency	Percentage %	Frequency	Percentage %
Negative	39	35.1	37	51.4	76	41.5
Positive	72	64.9	35	48.6	107	58.5
Total	111	100	72	100	183	100

Table (4.5) illustrate the distribution of TPOAb results among study group; over all (58.5%) were positive and (41.5%) were negative. In **hypothyroidism** (64.9%) were positive and the rest (35.1%) were negative, but in **hyperthyroidism** (48.6%) were positive and (51.4%) were negative.

Table (4.6): Thyroglobulin antibodies among test group

TgAb results	N= 111		N = 72		Total	
	Hypothyroidism		Hyperthyroidism			
	Frequency	Percentage %	Frequency	Percentage %	Frequency	Percentage %
Negative	60	54.1	50	69.5	110	60.1
Positive	51	45.9	22	30.5	73	39.9
Total	111	100	72	100	183	100

Table (4.6) revealed the distribution of TgAb results among study group; over all (39.9%) were positive and (60.1%) were negative. In **hypothyroidism** (45.9%) were positive and the rest (54.1%) were negative, but in **hyperthyroidism** (30.5%) were positive and (69.5%) were negative.

Table (4.7): Focal thyroid signs among the study group

Focal thyroid sings	N = 111				Total %	N = 72				Total %
	Hypothyroidism					Hyperthyroidism				
	Frequency		Percent%			Frequency		Percent%		
	Yes	No	Yes	No		Yes	No	Yes	No	
Solitary nodule	21	90	18.9	81.1	100	20	52	27.8	72.2	100
Multinodular	12	99	10.8	89.2	100	7	65	9.7	90.3	100
Diffuse	9	102	8.1	91.9	100	15	57	20.8	79.2	100
No goiter	69		62.2		100	29		40.3		100
Bruit	0	111	0	100	100	1	71	1.4	98.6	100

Table (4.7) depicted the distribution of study group according to Focal thyroid signs; **in hypothyroidism**; (solitary nodule 18.9%), (multinodular goiter 10.8%), (diffused goiter 8.1%), (no goiter 62.2%), **in hyperthyroidism**; (solitary nodule 27.8%), (multinodular 9.7%), (diffused 20.8%), (no goiter 40.3%), (bruit 1.4%).

Table (4.8): Hand signs among the study group

Hand sings	N = 111				Total	N = 72				Total
	Hypothyroidism					Hyperthyroidism				
	Frequency		Percent%			Frequency		Percent%		
	Yes	No	Yes	No		Yes	No	Yes	No	
Fine tremor	6	105	5.4	94.6	100%	49	23	68.1	31.9	100%
Sweating	3	108	2.7	97.3	100%	41	31	56.9	43.1	100%
Hotness	5	106	4.5	95.5	100%	47	25	65.3	34.7	100%

Table (4.8): identified the distribution of study group according to Hand signs; **in hypothyroidism;** (fine tremor 5.4%), (sweating 2.7%), (hotness 4.5%), **in hyperthyroidism**; (fine tremor 68.1%), (sweating 56.9%), (hotness 65.3%).

Table (4.9): Other diseases among the study group

Other diseases	N = 111				Total	N = 72				Total
	Hypothyroidism					Hyperthyroidism				
	Frequency		Percent%			Frequency		Percent%		
	Yes	No	Yes	No		Yes	No	Yes	No	
DM	11	100	9.9	90.1	100%	2	70	2.8	97.2	100%
Rheumatoid arthritis	1	110	0.9	99.1	100%	1	71	1.4	98.6	100%
Pregnancy	0	111	0	100	100%	1	71	1.4	98.6	100%
HTN	7	104	6.3	93.7	100%	2	70	2.8	97.2	100%

Table (4.9): provided the distribution of study group according to other diseases; **in hypothyroidism;** (diabetes mellitus 9.9%), (rheumatoid arthritis 0.9%), (hypertension 6.3%), **in hyperthyroidism**; (diabetes mellitus 2.8%), (rheumatoid arthritis 1.4%), (hypertension 2.8%).

Table (4.10): Correlation between TPOAb and thyroid hormones in hyperthyroidism patients

TPOAb Results	Frequency	Percent %	Mean of TSH	Mean of T4	Mean of FT4	Mean of T3	Mean of FT3
Negative (< 40 IU/ml)	37	51.4	1.22	135.3	28.9	2.8	7.3
Positive (>40IU/ml)	35	48.6	0.59	146.9	35.2	3.6	12.8
Total	72	100%					
P. value =			**0.122**	**0.488**	**0.300**	**0.147**	**0.032***

***** t- test P <0.05 is significant.

Table (4.10): stated the T- test of mean of TPO antibody with mean of thyroid hormones **in hyperthyroidism** patients; in **negative TPOAb** (TSH= 1.22, T4= 135.3, FT4= 28.9, T3=2.8, FT3=7.3), in **positive TPOAb** (TSH=0.59, T4=146.9, FT4=35.2, T3=3.6, FT3=12.8).

Table (4.11): Thyroid peroxidase antibody levels in hypothyroidism patients

TPOAb Results	Frequency	Percent %	Mean of TSH	Mean of T4	Mean of FT4	Mean of T3	Mean of FT3
Negative (< 40 IU/ml)	39	35.1	18.5	63.6	12.5	1.36	3.3
Positive (> 40IU/ml)	72	64.9	22.7	63.6	11.8	1.35	3.7
Total	**111**	**100%**					
P. value =			**0.561**	**0.999**	**0.513**	**0.874**	**0.029***

***** t- test P <0.05 is significant.

Table (4.11): summarized the T- test of mean of TPO antibody with mean of thyroid hormones **in hypothyroidism** patients; in **negative TPOAb** (TSH= 18.5, T4= 63.6, FT4= 12.5, T3=1.36, FT3=3.3), in **positive TPOAb** (TSH=22.7, T4=63.6, FT4=11.8, T3=1.35, FT3=3.7)

Table (4.12): Thyroglobulin antibody and thyroid hormones antibody means in hyperthyroidism patients

TgAb Results	Frequency	Percent %	Mean of TSH	Mean of T4	Mean of FT4	Mean of T3	Mean of FT3
Negative (< 125 IU/ml)	50	69.5	0.95	129.1	28.1	2.7	7.7
Positive (>125IU/ml)	22	30.5	0.82	167.8	40.8	4.2	15.4
Total	**72**	**100%**					
P. value =			**0.776**	**0.030***	**0.048***	**0.014***	**0.004****

***** t- test P <0.05 is significant.

Table (4.12): denoted the T- test of mean of **TgAb** antibody with mean of thyroid hormones **in hyperthyroidism** patients; in **negative TgAb** (TSH= 0.95, T4= 129.1, FT4= 28.1, T3=2.7, FT3=7.7), in **positive TgAb** (TSH=0.8, T4=167.8, FT4=40.8, T3=4.2, FT3=15.4)

Table (4.13): Thyroglobulin Antibody and thyroid hormones means in hypothyroidism patients

TgAb Results	Frequency	Percent %	Mean of TSH	Mean of T4	Mean of FT4	Mean of T3	Mean of FT3
Negative (< 125 IU/ml)	60	54.1	16.9	67.3	12.4	1.35	3.5
Positive (> 125 IU/ml)	51	45.9	26.3	59.2	11.7	1.37	3.7
Total	**111**	**100%**					
P. value =			**0.169**	**0.106**	**0.467**	**0.809**	**0.554**

***** t- test P <0.05 is significant.

Table (4.13): explained the T- test of mean of TgAb antibody with mean of thyroid hormones in hypothyroidism patients; in **negative TgAb** (TSH= 16.9, T4= 67.3, FT4= 12.4, T3=1.35, FT3=3.5), in **positive TgAb** (TSH=26.3, T4=59.2, FT4=11.7, T3=1.37, FT3=3.7)

Table (4.14): Comparison between serum TSH in test and control groups:

TSH						
Groups	Hypothyroidism			Hyperthyroidism		
	Frequency	Mean	Sig.(2-tailed)	Frequency	Mean	Sig.(2-tailed)
Test	111	21.19	**0.000****	72	0.9	**0.000****
Control	100	2.1		100	2.1	

***** t- test P <0.05 is significant.

Table (4.14): illustrated the comparison between mean of serum TSH in test groups and control group: the mean of TSH **in hypothyroidism** was (21.19) and in control group was (2.1) with (P.value 0.000), statistically highly significant difference between two means, **in hyperthyroidism**; the mean of TSH was (0.9) and in control group was (2.1) with (P.value 0.000).

Table (4.15): Correlation between serum TT_4 in test and control groups:

TT_4						
Groups	Hypothyroidism			Hyperthyroidism		
	Frequency	Mean	Sig.(2-tailed)	Frequency	Mean	Sig.(2-tailed)
Test	111	63.6	**0.000****	72	140.9	**0.000****
Control	100	93.1		100	93.1	

***** t- test P <0.05 is significant.

Table (4.15): presented the comparison between mean of serum **total T4** in test groups and control group: the mean of **T4** in **hypothyroidism** was (63.6) and in control group was (93.1) with mean of difference (29.5) and (P.value 0.000), statistically highly significant difference between two means, in **hyperthyroidism**; the mean of **T4** was (140.95) and in control group was (93.1) with mean difference of (47.8) and (P.value 0.000).

Table (4.16): Relationship between serum FT4 in test and control groups:

FT4						
Groups	Hypothyroidism			Hyperthyroidism		
	Frequency	Mean	Sig.(2-tailed)	Frequency	Mean	Sig.(2-tailed)
Test	111	12.1	**0.000****	72	31.97	**0.000****
Control	100	17.6		100	17.6	

***** t- test P <0.05 is significant.

Table (4.16): indicated the comparison between mean of serum **FT4** in test groups and control group: the mean of **FT4 in hypothyroidism** was (12.1) and in control group was (17.6) with mean difference of (5.5) and (P.value 0.000), statistically highly significant difference between two means, **in hyperthyroidism**; the mean of **FT4** was (31.97) and in control group was (17.6) with mean difference of (14.37) and (P.value 0.000).

Table (4.17): Difference between serum TT3 in test and control groups:

TT3						
Groups	Hypothyroidism			Hyperthyroidism		
	Frequency	Mean	Sig.(2-tailed)	Frequency	Mean	Sig.(2-tailed)
Test	111	1.17	**0.000****	72	3.36	**0.000****
Control	100	1.58		100	1.58	

***** t- test P <0.05 is significant.

Table (4.17): revealed the comparison between mean of serum **TT_3** in test groups and control group: the mean of **TT_3 in hypothyroidism** was (1.17) and in control group was (1.58) with (P.value 0.000), statistically highly significant difference between two means, **in hyperthyroidism**; the mean of **TT_3** was (3.36) and in control group was (1.58) with (P.value 0.000).

Table (4.18): Significance between serum FT_3 in test and control groups:

FT3						
Groups	**Hypothyroidism**			**Hyperthyroidism**		
	Frequency	**Mean**	**Sig.(2-tailed)**	**Frequency**	**Mean**	**Sig.(2-tailed)**
Test	111	3.58	**0.000****	72	10.04	**0.000****
Control	100	4.26		100	4.26	

***** t- test P <0.05 is significant.

Table (4.18): prevailed the comparison between mean of **serum FT3** in test groups and control group: the mean of **FT3 in hypothyroidism** was (3.58) and in control group was (4.26) with mean difference of (0.68) and (P.value 0.000), statistically highly significant difference between two means, **in hyperthyroidism**; the mean of **FT3** was (10.04) and in control group was (4.26) with mean difference of (5.78) and (P.value 0.000).

Table (4.19): Comparison between thyroid parameters in presence and absence of restlessness in hyperthyroidism patients

Thyroid parameter	Restlessness	Frequency	Percent %	Mean	Sig.(2-tailed)
TSH	Yes	64	88.9	0.85	0.419
	No	8	11.1	1.4	
TT4	Yes	64	88.9	144.0	0.304
	No	8	11.1	116.8	
FT4	Yes	64	88.9	33.5	0.162
	No	8	11.1	20.2	
TT3	Yes	64	88.9	3.31	0.148
	No	8	11.1	2.05	
FT3	Yes	64	88.9	10.8	0.104
	No	8	11.1	4.1	
TPOAb	Yes	64	88.9	128.2	0.333
	No	8	11.1	241.5	
TgAb	Yes	64	88.9	145.9	0.093
	No	8	11.1	401.0	

***** t- test P <0.05 is significant.

Table (4.19): showed the correlation between restlessness and the means of thyroid parameters **in hyperthyroidism patients**; there was no significant correlation between restlessness and thyroid parameters, and thyroid antibodies.

Table (4.20): Correlation between thyroid parameters in presence and absence of sweating in hyperthyroidism patients

Thyroid parameter	Sweating	Frequency	Percent %	Mean	Sig.(2-tailed)
TSH	Yes	57	79.2	0.8	0.490
	No	15	20.8	1.2	
TT4	Yes	57	79.2	147.4	0.128
	No	15	20.8	116.4	
FT4	Yes	57	79.2	34.8	0.063
	No	15	20.8	21.2	
TT3	Yes	57	79.2	3.37	0.163
	No	15	20.8	2.4	
FT3	Yes	57	79.2	11.3	0.067
	No	15	20.8	5.5	
TPOAb	Yes	57	79.2	93.9	0.011*
	No	15	20.8	318.9	
TgAb	Yes	57	79.2	124.3	0.04*
	No	15	20.8	364.2	

****** t- test P <0.005 is highly significant.

Table (4.20): predicted the correlation between sweating and the means of thyroid parameters **in hyperthyroidism patients;** there was no significant correlation between sweating and thyroid hormones, but there was significant correlation between sweating and **thyroid antibodies.**

Table (4.21): Comparison between thyroid parameters in presence and absence of diarrhea in hyperthyroidism patients

Thyroid parameter	Diarrhea	Frequency	Percent %	Mean	p.value
TSH	Yes	21	29.2	0.9	0.972
	No	51	70.8	0.92	
TT4	Yes	21	29.2	153.1	0.349
	No	51	70.8	135.9	
FT4	Yes	21	29.2	39.9	0.088
	No	51	70.8	28.7	
TT3	Yes	21	29.2	3.7	0.254
	No	51	70.8	3.0	
FT3	Yes	21	29.2	14.2	0.036*
	No	51	70.8	8.3	
TPOAb	Yes	21	29.2	49.9	0.111
	No	51	70.8	178.3	
TgAb	Yes	21	29.2	102.0	0.335
	No	51	70.8	204.0	

***** t- test P <0.05 is significant.

Table (4.21): demonstrated the correlation between Diarrhea and the means of thyroid parameters **in hyperthyroidism patients**; there was significant correlation between Diarrhea and **FT3**, but there was no significant correlation with other thyroid parameters and antibodies.

Table (4.22): Correlation between thyroid parameters in presence and absence of fatigue in hyperthyroidism patients

Thyroid parameter	Fatigue	Frequency	Percent %	Mean	p.value
TSH	Yes	30	41.7	1.02	0.648
	No	42	58.3	0.83	
TT4	Yes	30	41.7	143.1	0.827
	No	42	58.3	139.4	
FT4	Yes	30	41.7	31.4	0.886
	No	42	58.3	32.3	
TT3	Yes	30	41.7	3.0	0.628
	No	42	58.3	3.3	
FT3	Yes	30	41.7	9.3	0.622
	No	42	58.3	10.6	
TPOAb	Yes	30	41.7	235.5	0.027*
	No	42	58.3	73.2	
TgAb	Yes	30	41.7	212.3	0.505
	No	42	58.3	147.1	

***** t- test P <0.05 is significant.

Table (4.22): prevailed the correlation between fatigue and the means of thyroid parameters **in hyperthyroidism patients**; there was no significant correlation between fatigue and thyroid hormones, but there was statistically significant correlation between fatigue and **Anti-TPO antibody**.

Table (4.23): Association between thyroid parameters in presence & absence of weight loss in hyperthyroidism patients

Thyroid parameter	Weight loss	Frequency	Percent %	Mean	p.value
TSH	Yes	60	83.3	0.8	0.386
	No	12	16.7	1.3	
TT4	Yes	60	83.3	141.3	0.915
	No	12	16.7	139.0	
FT4	Yes	60	83.3	32.9	0.473
	No	12	16.7	27.1	
TT3	Yes	60	83.3	3.2	0.790
	No	12	16.7	3.0	
FT3	Yes	60	83.3	10.6	0.336
	No	12	16.7	7.3	
TPOAb	Yes	60	83.3	134.0	0.681
	No	12	16.7	174.7	
TgAb	Yes	60	83.3	126.1	0.023*
	No	12	16.7	415.2	

***** t- test P <0.05 is significant.

Table (4.23): estimated the correlation between weight loss and the means of thyroid parameters **in hyperthyroidism patients**; there was no significant correlation between weight loss and thyroid hormones, but there was statistically significant negative correlation between weight loss and **Anti-Tg antibody.**

Table (4.24): Correlation between thyroid parameters in presence and absence of increased appetites in hyperthyroidism patients

Thyroid parameter	Increase appetites	Frequency	Percent %	Mean	p.value
TSH	Yes	16	22.2	0.38	0.165
	No	56	77.8	1.06	
TT4	Yes	16	22.2	163.0	0.155
	No	56	77.8	134.7	
FT4	Yes	16	22.2	33.2	0.822
	No	56	77.8	31.6	
TT3	Yes	16	22.2	3.5	0.521
	No	56	77.8	3.1	
FT3	Yes	16	22.2	9.6	0.846
	No	56	77.8	10.2	
TPOAb	Yes	16	22.2	305.2	0.015*
	No	56	77.8	93.8	
TgAb	Yes	16	22.2	252.6	0.385
	No	56	77.8	151.9	

***** t- test P <0.05 is significant.

Table (4.24): indicated the correlation between increase appetites and the means of thyroid parameters **in hyperthyroidism patients**; there was no significant correlation between increase appetites and thyroid hormones, but there was statistically significant correlation between increase appetites and **Anti-TPO antibody.**

Table (4.25): Association between thyroid parameters in presence and absence of fever in hyperthyroidism patients

Thyroid parameter	Fever	Frequency	Percent %	Mean	p.value
TSH	Yes	13	18.1	0.33	0.181
	No	59	81.9	1.04	
TT4	Yes	13	18.1	156.1	0.392
	No	59	81.9	137.3	
FT4	Yes	13	18.1	37.3	0.407
	No	59	81.9	30.8	
TT3	Yes	13	18.1	3.5	0.540
	No	59	81.9	3.1	
FT3	Yes	13	18.1	12.2	0.430
	No	59	81.9	9.6	
TPOAb	Yes	13	18.1	335.7	0.011*
	No	59	81.9	97.9	
TgAb	Yes	13	18.1	449.9	0.006*
	No	59	81.9	113.5	

***** t- test P <0.05 is significant.

Table (4.25): presented the correlation between fever and the means of thyroid parameters **in hyperthyroidism patients**; there was no statistically significant correlation between **fever and thyroid hormones**, but there was statistically significant correlation between fever and **thyroid antibodies.**

Table (4.26): Correlation between thyroid parameters in presence and absence of anorexia in hyperthyroidism patients

Thyroid parameter	Anorexia	Frequency	Percent %	Mean	p.value
TSH	Yes	16	22.2	1.2	0.484
	No	56	77.8	0.8	
TT4	Yes	16	22.2	135.8	0.741
	No	56	77.8	142.4	
FT4	Yes	16	22.2	26.0	0.290
	No	56	77.8	33.7	
TT3	Yes	16	22.2	2.9	0.555
	No	56	77.8	3.3	
FT3	Yes	16	22.2	8.2	0.450
	No	56	77.8	10.6	
TPOAb	Yes	16	22.2	209.7	0.316
	No	56	77.8	121.1	
TgAb	Yes	16	22.2	363.7	0.033*
	No	56	77.8	120.2	

***** t- test P <0.05 is significant.

Table (4.26): demoted the correlation between anorexia and the means of thyroid parameters **in hyperthyroidism patients**; there was no statistically significant correlation between **anorexia and thyroid hormones**, but there was significant correlation with **anti-Tg antibodies.**

Table (4.27): Comparison between thyroid parameters in presence & absence of exophthalmoses in hyperthyroidism patients

Thyroid parameter	Exophthalmoses	Frequency	Percent %	Mean	p.value
TSH	Yes	7	9.7	0.5	0.553
	No	65	90.3	1.0	
TT4	Yes	7	9.7	152.1	0.662
	No	65	90.3	139.8	
FT4	Yes	7	9.7	27.4	0.615
	No	65	90.3	32.5	
TT3	Yes	7	9.7	3.9	0.403
	No	65	90.3	3.1	
FT3	Yes	7	9.7	8.4	0.672
	No	65	90.3	10.2	
TPOAb	Yes	7	9.7	181.8	0.716
	No	65	90.3	136.4	
TgAb	Yes	7	9.7	402.0	0.118
	No	65	90.3	149.8	

***** t- test P <0.05 is significant.

Table (4.27): illustrated the correlation between exophthalmoses and the means of thyroid parameters **in hyperthyroidism patients**; there was no statistically significant correlation between **exophthalmoses** and **thyroid hormones, and thyroid antibodies.**

Table (4.28): Correlation between thyroid parameters in presence & absence of exophthalmoplagia in hyperthyroidism patients

Thyroid parameter	Exophthalmoplagia	Frequency	Percent %	Mean	p.value
TSH	Yes	5	6.9	0.2	0.362
	No	67	93.1	1.0	
TT4	Yes	5	6.9	180.7	0.190
	No	67	93.1	138.0	
FT4	Yes	5	6.9	43.2	0.304
	No	67	93.1	31.1	
TT3	Yes	5	6.9	4.8	0.099
	No	67	93.1	3.1	
FT3	Yes	5	6.9	18.7	0.066
	No	67	93.1	9.4	
TPOAb	Yes	5	6.9	140.2	0.996
	No	67	93.1	140.9	
TgAb	Yes	5	6.9	552.3	0.030*
	No	67	93.1	146.1	

***** t- test P <0.05 is significant.

Table (4.28): provided the correlation between exophthalmoplagia and the means of thyroid parameters **in hyperthyroidism patients**; there was no statistically significant correlation between **exophthalmoplagia and thyroid hormones**, but there was significant correlation with **anti-Tg antibodies.**

Table (4.29): Association between thyroid parameters in presence and absence of loss of eye brow in hyperthyroidism patients

Thyroid parameter	Loss of eye brow	Frequency	Percent %	Mean	p.value
TSH	Yes	2	2.8	2.8	0.127
	No	70	97.2	0.9	
TT4	Yes	2	2.8	102.2	0.431
	No	70	97.2	142.1	
FT4	Yes	2	2.8	19.8	0.493
	No	70	97.2	32.3	
TT3	Yes	2	2.8	1.4	0.276
	No	70	97.2	3.2	
FT3	Yes	2	2.8	3.5	0.390
	No	70	97.2	10.2	
TPOAb	Yes	2	2.8	14.9	0.564
	No	70	97.2	144.4	
TgAb	Yes	2	2.8	48.3	0.659
	No	70	97.2	177.9	

***** t- test P <0.05 is significant.

Table (4.29): clarified the correlation between loss of eye brow and the means of thyroid parameters **in hyperthyroidism patients**; there was no statistically significant correlation between **loss of eye brow, thyroid hormones, and thyroid antibodies.**

Table (4.30): Association between thyroid parameters in presence and absence of thick skin in hyperthyroidism patients

Thyroid parameter	Thick skin	Frequency	Percent %	Mean	p.value
TSH	Yes	7	9.7	1.2	0.608
	No	65	90.3	0.9	
TT4	Yes	7	9.7	153.5	0.622
	No	65	90.3	139.6	
FT4	Yes	7	9.7	34.4	0.789
	No	65	90.3	31.7	
TT3	Yes	7	9.7	2.5	0.443
	No	65	90.3	3.2	
FT3	Yes	7	9.7	8.7	0.735
	No	65	90.3	10.2	
TPOAb	Yes	7	9.7	222.2	0.468
	No	65	90.3	132.0	
TgAb	Yes	7	9.7	487.2	0.031*
	No	65	90.3	140.6	

***** t- test P <0.05 is significant.

Table (4.30): presented correlation between thick skin and the means of thyroid parameters **in hyperthyroidism patients;** there was no statistically significant correlation between **thick skin and thyroid hor**mones, but there was significant correlation with **anti-Tg antibodies.**

Table (4.31): Relationship between thyroid parameters in presence and absence of pretibial myexodema in hyperthyroidism patients

Thyroid parameter	Pretibial myexodema	Frequency	Percent %	Mean	p.value
TSH	Yes	3	4.2	0.01	0.359
	No	69	95.8	0.95	
TT4	Yes	3	4.2	295.5	0.000**
	No	69	95.8	134.2	
FT4	Yes	3	4.2	60.6	0.044*
	No	69	95.8	30.7	
TT3	Yes	3	4.2	8.1	0.000**
	No	69	95.8	2.9	
FT3	Yes	3	4.2	26.1	0.008*
	No	69	95.8	9.3	
TPOAb	Yes	3	4.2	336.8	0.266
	No	69	95.8	132.3	
TgAb	Yes	3	4.2	181.3	0.976
	No	69	95.8	174.0	

***** t- test P <0.05 is significant.

Table (4.31): revealed the correlation between pretibial myexodema and the means of thyroid parameters **in hyperthyroidism patients;** there was no statistically significant correlation between **pretibial myexodema and TSH, anti-TPO, and anti-Tg**, but there was significant correlation with **TT4, FT4, TT3, and FT3.**

Table (4.32): Comparison between thyroid parameters in presence and absence of fine tremor in hyperthyroidism patients

Thyroid parameter	Fine tremor	Frequency	Percent %	Mean	p.value
TSH	Yes	49	68.1	0.8	0.234
	No	23	31.9	1.3	
TT4	Yes	49	68.1	149.7	0.122
	No	23	31.9	122.3	
FT4	Yes	49	68.1	34.3	0.255
	No	23	31.9	27.0	
TT3	Yes	49	68.1	3.46	0.127
	No	23	31.9	2.56	
FT3	Yes	49	68.1	11.5	0.082
	No	23	31.9	6.7	
TPOAb	Yes	49	68.1	261.8	0.022*
	No	23	31.9	84.0	
TgAb	Yes	49	68.1	123.5	0.121
	No	23	31.9	282.5	

***** t- test P <0.05 is significant.

Table (4.32): stated the correlation between fine tremor and the means of thyroid parameters **in hyperthyroidism patients;** there was no statistically significant correlation between **fine tremor and thyroid hormones**, but there was significant correlation with **Anti-TPO antibodies.**

Table (4.33): Correlation between thyroid parameters in presence and absence of sweating of hands in hyperthyroidism patients

Thyroid parameter	Sweating of hands	Frequency	Percent %	Mean	p.value
TSH	Yes	41	56.9	0.7	0.141
	No	31	43.1	1.26	
TT4	Yes	41	56.9	152.5	0.107
	No	31	43.1	125.6	
FT4	Yes	41	56.9	35.4	0.188
	No	31	43.1	27.4	
TT3	Yes	41	56.9	3.4	0.432
	No	31	43.1	2.9	
FT3	Yes	41	56.9	11.9	0.084
	No	31	43.1	7.5	
TPOAb	Yes	41	56.9	253.0	0.007*
	No	31	43.1	55.9	
TgAb	Yes	41	56.9	284.2	0.045*
	No	31	43.1	91.2	

***** t- test P <0.05 is significant.

Table (4.33): reflected the correlation between sweating of hands and the means of thyroid parameters **in hyperthyroidism patients**; there was no statistically significant correlation between **sweating of hands and thyroid hormones**, but there was significant correlation between **sweating of hands, Anti-TPO and Anti-Tg antibodies.**

Table (4.34): Association between thyroid parameters in presence and absence of hotness in hyperthyroidism patients

Thyroid parameter	Hotness	Frequency	Percent %	Mean	p.value
TSH	Yes	47	65.3	0.8	0.579
	No	25	34.7	1.1	
TT4	Yes	47	65.3	150.9	0.096
	No	25	34.7	122.1	
FT4	Yes	47	65.3	36.1	0.057
	No	25	34.7	24.2	
TT3	Yes	47	65.3	3.5	0.058
	No	25	34.7	2.4	
FT3	Yes	47	65.3	12. 1	0.028*
	No	25	34.7	6.2	
TPOAb	Yes	47	65.3	131.2	0.722
	No	25	34.7	158.8	
TgAb	Yes	47	65.3	129.4	0.200
	No	25	34.7	258.5	

***** t- test P <0.05 is significant.

Table (4.34): denoted the correlation between hotness and the means of thyroid parameters **in hyperthyroidism patients**; there was no statistically significant correlation between **hotness, thyroid hormones and thyroid antibodies**, but there was significant correlation between **hotness and fT3.**

Table (4.35): Relationship between thyroid parameters in presence and absence of tachycardia in hyperthyroidism patients

Thyroid parameter	Tachycardia	Frequency	Percent %	Mean	p.value
TSH	Yes	28	38.9	0.7	0.418
	No	44	61.1	1.0	
TT4	Yes	28	38.9	156.9	0.123
	No	44	61.1	130.7	
FT4	Yes	28	38.9	34.3	0.521
	No	44	61.1	30.4	
TT3	Yes	28	38.9	3.9	0.046*
	No	44	61.1	2.7	
FT3	Yes	28	38.9	12.6	0.106
	No	44	61.1	8.3	
TPOAb	Yes	28	38.9	177.5	0.426
	No	44	61.1	117.4	
TgAb	Yes	28	38.9	183.8	0.874
	No	44	61.1	168.1	

***** t- test P <0.05 is significant.

Table (4.35): predicted the correlation between tachycardia and the means of thyroid parameters **in hyperthyroidism patients**; there was no statistically significant correlation between **tachycardia, thyroid hormones and thyroid antibodies** but there was significant correlation with **TT3.**

Table (4.36): Comparison between thyroid parameters in presence and absence of bradycardia in hyperthyroidism patients

Thyroid parameter	Bradycardia	Frequency	Percent %	Mean	p.value
TSH	Yes	2	2.8	0.01	0.458
	No	70	97.2	0.9	
TT4	Yes	2	2.8	168.1	0.582
	No	70	97.2	140.2	
FT4	Yes	2	2.8	23.4	0.630
	No	70	97.2	32.2	
TT3	Yes	2	2.8	3.3	0.963
	No	70	97.2	3.2	
FT3	Yes	2	2.8	10.2	0.978
	No	70	97.2	10.0	
TPOAb	Yes	2	2.8	52.8	0.687
	No	70	97.2	143.3	
TgAb	Yes	2	2.8	223.4	0.863
	No	70	97.2	172.8	

***** t- test P <0.05 is significant.

Table (4.36): identified the correlation between bradycardia and the means of thyroid parameters **in hyperthyroidism patients**; there was no statistically significant correlation between **bradycardia, thyroid hormones and thyroid antibodies.**

Table (4.37): Correlation between thyroid parameters with newly discovered and old cases in hyperthyroidism patients

Thyroid parameter	Cases	Frequency	Percent %	Mean	p.value
TSH	Old	42	58.3	1.34	0.012*
	New	30	41.7	0.3	
TT4	Old	42	58.3	102.5	0.000*
	New	30	41.7	194.7	
FT4	Old	42	58.3	20.3	0.000*
	New	30	41.7	48.2	
TT3	Old	42	58.3	2.1	0.000*
	New	30	41.7	4.6	
FT3	Old	42	58.3	5.2	0.000*
	New	30	41.7	16.7	
TPOAb	Old	42	58.3	112.4	0.361
	New	30	41.7	180.5	
TgAb	Old	42	58.3	169.0	0.899
	New	30	41.7	181.5	

***** t- test P <0.05 is significant.

Table (4.37): reflected the correlation between type of cases and the means of thyroid parameters **in hyperthyroidism patients**; there was statistically highly significant correlation between **newly discovered cases and thyroid hormones.**

Table (4.38): Relationship between thyroid parameters in presence and absence of family history in hyperthyroidism patients

Thyroid parameter	Family History	Frequency	Percent %	Mean	p.value
TSH	Yes	45	62.5	0.82	0.585
	No	27	37.5	1.0	
TT4	Yes	45	62.5	145.1	0.518
	No	27	37.5	134.0	
FT4	Yes	45	62.5	32.8	0.717
	No	27	37.5	30.5	
TT3	Yes	45	62.5	3.4	0.131
	No	27	37.5	2.6	
FT3	Yes	45	62.5	11.4	0.171
	No	27	37.5	7.7	
TPOAb	Yes	45	62.5	131.5	0.746
	No	27	37.5	156.2	
TgAb	Yes	45	62.5	122.1	0.160
	No	27	37.5	261.1	

***** t- test P <0.05 is significant.

Table (4.38): indicated the correlation between family history and the means of thyroid parameters **in hyperthyroidism patients**; there was no significant correlation between **family history, thyroid hormones and thyroid antibodies.**

Table (4.39): Comparison between thyroid parameters with first and second degree family history in hyperthyroidism patients

Thyroid parameter	Degree of F.H	Frequency	Percent %	Mean	p.value
TSH	First degree	19	70.4	1.1	0.705
	Second degree	8	29.6	0.8	
TT4	First degree	19	70.4	115.6	0.015*
	Second degree	8	29.6	177.5	
FT4	First degree	19	70.4	22.3	0.013*
	Second degree	8	29.6	50.0	
TT3	First degree	19	70.4	2.0	0.008*
	Second degree	8	29.6	3.9	
FT3	First degree	19	70.4	5.0	0.004**
	Second degree	8	29.6	14.3	
TPOAb	First degree	19	70.4	197.6	0.243
	Second degree	8	29.6	57.8	
TgAb	First degree	19	70.4	333.6	0.283
	Second degree	8	29.6	88.8	

***** t- test P <0.05 is significant.

Table (4.39): adopted the correlation between degree of family history and the means of thyroid parameters **in hyperthyroidism patients**; there was statistically highly significant correlation between **degree of family history and thyroid hormones.**

Table (4.40): Correlation between thyroid parameters in presence and absence of family history in hypothyroidism patients

Thyroid parameter	Family History	Frequency	Percent %	Mean	p.value
TSH	No	74	66.7	23.6	0.300
	Yes	37	33.3	16.1	
TT4	No	74	66.7	61.9	0.344
	Yes	37	33.3	66.9	
FT4	No	74	66.7	11.8	0.589
	Yes	37	33.3	12.4	
TT3	No	74	66.7	1.3	0.231
	Yes	37	33.3	1.4	
FT3	No	74	66.7	3.4	0.073
	Yes	37	33.3	3.8	
TPOAb	No	74	66.7	162.2	0.306
	Yes	37	33.3	207.0	
TgAb	No	74	66.7	335.4	0.734
	Yes	37	33.3	376.7	

***** t- test P <0.05 is significant.

Table (4.40): predicted the correlation between family history and the means of thyroid parameters **in hypothyroidism patients**; there was no significant correlation between **family history, thyroid hormones and thyroid antibodies.**

Table (4.41): Relationship between thyroid parameters and family history degree in hypothyroidism patients

Thyroid parameter	Degree of F.H	Frequency	Percent %	Mean	p.value
TSH	First degree	24	64.9	19.4	0.339
	Second degree	13	35.1	10.2	
TT4	First degree	24	64.9	63.4	0.210
	Second degree	13	35.1	73.4	
FT4	First degree	24	64.9	12.0	0.498
	Second degree	13	35.1	13.1	
TT3	First degree	24	64.9	1.4	0.843
	Second degree	13	35.1	1.4	
FT3	First degree	24	64.9	3.9	0.289
	Second degree	13	35.1	3.6	
TPOAb	First degree	24	64.9	262.7	0.065
	Second degree	13	35.1	104.0	
TgAb	First degree	24	64.9	453.6	0.326
	Second degree	13	35.1	234.8	

***** t- test P <0.05 is significant.

Table (4.41): demonstrated the correlation between degree of family history and the means of thyroid parameters **in hypothyroidism patients**; there was no significant correlation between **degree of family history, thyroid hormones and thyroid antibodies.**

Table (4.42): Association between thyroid parameters in presence and absence of restlessness in hypothyroidism patients

Thyroid parameter	Restlessness	Frequency	Percent %	Mean	p.value
TSH	Yes	12	10.8	7.1	0.151
	No	99	89.2	22.8	
TT4	Yes	12	10.8	64.9	0.849
	No	99	89.2	63.4	
FT4	Yes	12	10.8	13.8	0.212
	No	99	89.2	11.8	
TT3	Yes	12	10.8	1.4	0.678
	No	99	89.2	1.3	
FT3	Yes	12	10.8	4.2	0.029*
	No	99	89.2	3.5	
TPOAb	Yes	12	10.8	248.9	0.225
	No	99	89.2	168.4	
TgAb	Yes	12	10.8	430.4	0.621
	No	99	89.2	339.3	

***** t- test P <0.05 is significant.

Table (4.42): summarized the correlation between restlessness and the means of thyroid parameters **in hypothyroidism patients**; there was no significant correlation **between restlessness** and **thyroid hormones and thyroid antibodies, except with FT3.**

Table (4.43): Correlation between thyroid parameters in presence and absence of diarrhea in hypothyroidism patients

Thyroid parameter	Diarrhea	Frequency	Percent %	Mean	p.value
TSH	Yes	3	2.7	9.3	0.565
	No	108	97.3	21.5	
TT4	Yes	3	2.7	68.2	0.757
	No	108	97.3	63.4	
FT4	Yes	3	2.7	13.4	0.641
	No	108	97.3	12.0	
TT3	Yes	3	2.7	1.4	0.960
	No	108	97.3	1.4	
FT3	Yes	3	2.7	4.4	0.198
	No	108	97.3	3.6	
TPOAb	Yes	3	2.7	431.8	0.038*
	No	108	97.3	170.0	
TgAb	Yes	3	2.7	676.9	0.340
	No	108	97.3	340.1	

***** t- test P <0.05 is significant.

Table (4.43): identified the correlation between diarrhea and the means of thyroid parameters **in hypothyroidism patients**; there was no significant correlation between **diarrhea, thyroid hormones and thyroid antibodies,** but there was significant correlation **with anti-TPO.**

Table (4.44): Relationship between thyroid parameters in presence and absence of constipation in hypothyroidism patients

Thyroid parameter	Constipation	Frequency	Percent %	Mean	p.value
TSH	Yes	59	53.1	25.2	0.208
	No	52	46.2	16.6	
TT4	Yes	59	53.1	60.3	0.164
	No	52	46.2	67.3	
FT4	Yes	59	53.1	10.9	0.012*
	No	52	46.2	13.3	
TT3	Yes	59	53.1	1.3	0.084
	No	52	46.2	1.4	
FT3	Yes	59	53.1	3.3	0.005**
	No	52	46.2	3.8	
TPOAb	Yes	59	53.1	151.3	0.182
	No	52	46.2	206.4	
TgAb	Yes	59	53.1	388.8	0.461
	No	52	46.2	304.2	

***** t- test P <0.05 is significant.

Table (4.44): illustrated the correlation between constipation and the means of thyroid parameters **in hypothyroidism patients**; there was no significant correlation between **constipation, thyroid hormones and thyroid antibodies,** but statistically significant correlation **with free thyroid hormones.**

Table (4.45): Comparison between thyroid parameters in presence and absence of fatigue in hypothyroidism patients

Thyroid parameter	Fatigue	Frequency	Percent %	Mean	p.value
TSH	Yes	68	61.3	27.0	0.030*
	No	43	38.7	11.9	
TT4	Yes	68	61.3	59.0	0.021*
	No	43	38.7	70.7	
FT4	Yes	68	61.3	10.9	0.004**
	No	43	38.7	13.8	
TT3	Yes	68	61.3	1.3	0.048*
	No	43	38.7	1.4	
FT3	Yes	68	61.3	3.4	0.080
	No	43	38.7	3.8	
TPOAb	Yes	68	61.3	195.8	0.255
	No	43	38.7	147.6	
TgAb	Yes	68	61.3	429.2	0.077
	No	43	38.7	222.6	

***** t- test P <0.05 is significant.

Table (4.45): reviewed the correlation between fatigue and the means of thyroid parameters **in hypothyroidism patients**; there was significant correlation between **fatigue and thyroid hormones** and there was no significant correlation **with thyroid antibodies.**

Table (4.46): Association between thyroid parameters in presence and absence of heat intolerance in hypothyroidism patients

Thyroid parameter	Heat intolerance	Frequency	Percent %	Mean	p.value
TSH	Yes	6	5.4	9.7	0.424
	No	105	94.6	21.8	
TT4	Yes	6	5.4	61.5	0.842
	No	105	94.6	63.7	
FT4	Yes	6	5.4	13.3	0.540
	No	105	94.6	12.0	
TT3	Yes	6	5.4	1.3	0.899
	No	105	94.6	1.4	
FT3	Yes	6	5.4	4.2	0.120
	No	105	94.6	3.5	
TPOAb	Yes	6	5.4	358.2	0.035*
	No	105	94.6	166.8	
TgAb	Yes	6	5.4	1048.9	0.003**
	No	105	94.6	309.2	

***** t- test P <0.05 is significant.

Table (4.46): described the correlation between heat intolerance and the means of thyroid parameters **in hypothyroidism patients**; there was no significant correlation between **heat intolerance and thyroid hormones** but there was highly significant correlation **with thyroid antibodies.**

Table (4.47): Correlation between thyroid parameters in presence and absence of cold intolerance in hypothyroidism patients

Thyroid parameter	Cold intolerance	Frequency	Percent %	Mean	p.value
TSH	Yes	28	25.2	22.2	0.855
	No	83	74.8	20.8	
TT4	Yes	28	25.2	60.8	0.525
	No	83	74.8	64.5	
FT4	Yes	28	25.2	11.8	0.796
	No	83	74.8	12.1	
TT3	Yes	28	25.2	1.2	0.124
	No	83	74.8	1.4	
FT3	Yes	28	25.2	3.2	0.031*
	No	83	74.8	3.7	
TPOAb	Yes	28	25.2	188.6	0.747
	No	83	74.8	173.2	
TgAb	Yes	28	25.2	443.3	0.340
	No	83	74.8	317.5	

***** t- test P <0.05 is significant.

Table (4.47): revealed the correlation between weight loss and the means of thyroid parameters **in hypothyroidism patients**; there was no significant correlation between **weight loss, thyroid hormones and thyroid antibodies,** but there was significant correlation **with fT3.**

Table (4.48): Correlation between thyroid parameters in presence and absence of loss of eye brow in hypothyroidism patients

Thyroid parameter	Loss of eye brow	Frequency	Percent %	Mean	p.value
TSH	Yes	38	34.2	16.3	0.298
	No	73	65.8	23.7	
TT4	Yes	38	34.2	63.8	0.953
	No	73	65.8	63.4	
FT4	Yes	38	34.2	12.8	0.275
	No	73	65.8	11.6	
TT3	Yes	38	34.2	1.3	0.599
	No	73	65.8	1.4	
FT3	Yes	38	34.2	3.4	0.449
	No	73	65.8	3.6	
TPOAb	Yes	38	34.2	173.2	0.891
	No	73	65.8	179.1	
TgAb	Yes	38	34.2	413.2	0.419
	No	73	65.8	315.8	

***** t- test P <0.05 is significant.

Table (4.48): determined the correlation between loss of eye brow and the means of thyroid parameters **in hypothyroidism patients**; there was no significant correlation between **loss of eye brow, thyroid hormones and thyroid antibodies.**

Table (4.49): Association between thyroid parameters in presence and absence of proximal myopathy in hypothyroidism patients

Thyroid parameter	Proximal myopathy	Frequency	Percent %	Mean	p.value
TSH	Yes	76	68.5	23.1	0.403
	No	35	31.5	16.9	
TT4	Yes	76	68.5	62.6	0.582
	No	35	31.5	65.6	
FT4	Yes	76	68.5	11.7	0.359
	No	35	31.5	12.7	
TT3	Yes	76	68.5	1.3	0.046*
	No	35	31.5	1.4	
FT3	Yes	76	68.5	3.4	0.014*
	No	35	31.5	3.9	
TPOAb	Yes	76	68.5	180.8	0.790
	No	35	31.5	169.0	
TgAb	Yes	76	68.5	305.7	0.262
	No	35	31.5	443.6	

***** t- test P <0.05 is significant.

Table (4.49): identified the correlation between proximal myopathy and the means of thyroid parameters **in hypothyroidism patients**; there was no significant correlation between **weight, thyroid hormones and thyroid antibodies,** except significant correlation **with TT3, FT3.**

Table (4.50): Relationship between thyroid parameters in presence and absence of unexpressive face in hypothyroidism patients

Thyroid parameter	Unexpressive face	Frequency	Percent %	Mean	p.value
TSH	Yes	78	70.3	22.8	0.450
	No	33	29.7	17.2	
TT4	Yes	78	70.3	62.1	0.365
	No	33	29.7	67.0	
FT4	Yes	78	70.3	11.9	0.645
	No	33	29.7	12.4	
TT3	Yes	78	70.3	1.3	0.094
	No	33	29.7	1.4	
FT3	Yes	78	70.3	3.5	0.286
	No	33	29.7	3.7	
TPOAb	Yes	78	70.3	182.2	0704
	No	33	29.7	165.0	
TgAb	Yes	78	70.3	371.3	0.553
	No	33	29.7	296.9	

***** t- test P <0.05 is significant.

Table (4.50): presented the correlation between unexpressive face and the means of thyroid parameters **in hypothyroidism patients**; there was no significant correlation between **unexpressive face, thyroid hormones and thyroid antibodies.**

Table (4.51): Comparison between thyroid parameters in presence and absence of thick skin in hypothyroidism patients

Thyroid parameter	Thick skin	Frequency	Percent %	Mean	p.value
TSH	Yes	80	72.1	22.6	0.496
	No	31	27.9	17.4	
TT4	Yes	80	72.1	62.3	0.407
	No	31	27.9	66.9	
FT4	Yes	80	72.1	12.0	0.940
	No	31	27.9	12.1	
TT3	Yes	80	72.1	1.3	0.184
	No	31	27.9	1.4	
FT3	Yes	80	72.1	3.5	0.198
	No	31	27.9	3.8	
TPOAb	Yes	80	72.1	191.8	0.251
	No	31	27.9	139.1	
TgAb	Yes	80	72.1	407.1	0.103
	No	31	27.9	199.8	

***** t- test P <0.05 is significant.

Table (4.51): reviewed the correlation between thick skin and the means of thyroid parameters **in hypothyroidism patients**; there was no significant correlation between **thick skin, thyroid hormones and thyroid antibodies.**

Table (4.52): Correlation between thyroid parameters in presence and absence of slow relax reflex in hypothyroidism patients

Thyroid parameter	Slow relax reflex	Frequency	Percent %	Mean	p.value
TSH	Yes	8	7.2	73.1	0.000**
	No	103	92.8	17.1	
TT4	Yes	8	7.2	23.7	0.000**
	No	103	92.8	66.7	
FT4	Yes	8	7.2	4.7	0.000**
	No	103	92.8	12.6	
TT3	Yes	8	7.2	0.6	0.000**
	No	103	92.8	1.4	
FT3	Yes	8	7.2	2.2	0.000**
	No	103	92.8	3.6	
TPOAb	Yes	8	7.2	115.7	0.407
	No	103	92.8	181.9	
TgAb	Yes	8	7.2	542.5	0.346
	No	103	92.8	334.1	

***** t- test P <0.05 is significant.

Table (4.52): described the correlation between slow relax reflex and the means of thyroid parameters **in hypothyroidism patients**; there was highly significant correlation between **slow relax reflex and thyroid hormones** and without significant correlation **with thyroid antibodies.**

Table (4.53): Association between thyroid parameters in presence and absence of change in voice in hypothyroidism patients

Thyroid parameter	Change in voice	Frequency	Percent %	Mean	p.value
TSH	Yes	4	3.6	62.7	0.017*
	No	107	96.4	19.6	
TT4	Yes	4	3.6	40.5	0.074
	No	107	96.4	64.4	
FT4	Yes	4	3.6	9.2	0.265
	No	107	96.4	12.1	
TT3	Yes	4	3.6	0.9	0.020*
	No	107	96.4	1.3	
FT3	Yes	4	3.6	2.5	0.034*
	No	107	96.4	3.6	
TPOAb	Yes	4	3.6	212.3	0.742
	No	107	96.4	175.8	
TgAb	Yes	4	3.6	1016.1	0.023*
	No	107	96.4	324. 3	

***** t- test P <0.05 is significant.

****** t- test P <0.005 is highly significant.

Table (4.53): illustrated the correlation between change in voice and the means of thyroid parameters **in hypothyroidism patients**; there was significant correlation between **change in voice, TSH, TT3, FT3, and anti-Tg.**

Table (4.54): Relationship between thyroid parameters in presence and absence of solitary nodule in hypothyroidism patients

Thyroid parameter	Solitary nodule	Frequency	Percent %	Mean	p.value
TSH	Yes	21	18.9	12.7	0.233
	No	90	81.1	23.1	
TT4	Yes	21	18.9	63.1	0.938
	No	90	81.1	63.6	
FT4	Yes	21	18.9	12.3	0.805
	No	90	81.1	12.0	
TT3	Yes	21	18.9	1.4	0.473
	No	90	81.1	1.3	
FT3	Yes	21	18.9	3.6	0.609
	No	90	81.1	3.5	
TPOAb	Yes	21	18.9	241.5	0.130
	No	90	81.1	162.1	
TgAb	Yes	21	18.9	238.3	0.349
	No	90	81.1	375.0	

***** t- test P <0.05 is significant.

****** t- test P <0.005 is highly significant.

Table (4.54): pointed out the correlation between solitary nodule and the means of thyroid parameters **in hypothyroidism patients**; there was no significant correlation between **solitary nodule, thyroid hormones and thyroid antibodies.**

Table (4.55): Comparison between thyroid parameters in presence and absence of multinodular goiter in hypothyroidism patients

Thyroid parameter	Multinodular goiter	Frequency	Percent %	Mean	p.value
TSH	Yes	12	10.8	7.2	0.154
	No	99	89.2	22.8	
TT4	Yes	12	10.8	74.7	0.122
	No	99	89.2	62.2	
FT4	Yes	12	10.8	13.1	0.425
	No	99	89.2	11.9	
TT3	Yes	12	10.8	1.5	0.170
	No	99	89.2	1.3	
FT3	Yes	12	10.8	3.8	0.360
	No	99	89.2	3.5	
TPOAb	Yes	12	10.8	126.0	0.389
	No	99	89.2	183.3	
TgAb	Yes	12	10.8	454.4	0.522
	No	99	89.2	336.4	

***** t- test P <0.05 is significant.

****** t- test P <0.005 is highly significant.

Table (4.55): stated out the correlation between multinodular goiter and the means of thyroid parameters **in hypothyroidism patients**; there was no significant correlation between **multinodular goiter, thyroid hormones and thyroid antibodies.**

Table (4.56): Correlation between thyroid parameters in presence and absence of diffuse goiter in hypothyroidism patients

Thyroid parameter	Diffuse	Frequency	Percent %	Mean	p.value
TSH	Yes	9	8.1	17.1	0.727
	No	102	91.9	21.5	
TT4	Yes	9	8.1	68.8	0.535
	No	102	91.9	63.1	
FT4	Yes	9	8.1	11.5	0.743
	No	102	91.9	12.1	
TT3	Yes	9	8.1	1.35	0.922
	No	102	91.9	1.36	
FT3	Yes	9	8.1	3.4	0.772
	No	102	91.9	3.5	
TPOAb	Yes	9	8.1	263.6	0.213
	No	102	91.9	169.5	
TgAb	Yes	9	8.1	484.4	0.483
	No	102	91.9	337.2	

***** t- test P <0.05 is significant.

****** t- test P <0.005 is highly significant.

Table (4.56): assessed the correlation between diffused goiter and the means of thyroid parameters **in hypothyroidism patients**; there was no significant correlation between **diffused goiter, thyroid hormones and thyroid antibodies.**

Table (4.57): Association between thyroid parameters in presence and absence of bradycardia in hypothyroidism patients

Thyroid parameter	Bradycardia	Frequency	Percent %	Mean	p.value
TSH	Yes	5	4.5	53.9	0.036*
	No	106	95.5	19.6	
TT4	Yes	5	4.5	44.2	0.093
	No	106	95.5	64.5	
FT4	Yes	5	4.5	10.5	0.488
	No	106	95.5	12.1	
TT3	Yes	5	4.5	1.0	0.057
	No	106	95.5	1.3	
FT3	Yes	5	4.5	2.8	0.098
	No	106	95.5	3.6	
TPOAb	Yes	5	4.5	134.3	0.653
	No	106	95.5	179.1	
TgAb	Yes	5	4.5	93.3	0.331
	No	106	95.5	361.3	

***** t- test P <0.05 is significant.

Table (4.57): pointed out the correlation between weight loss and the means of thyroid parameters **in hypothyroidism patients**; there was no significant correlation between **weight loss, thyroid hormones and thyroid antibodies, except TSH.**

Table (4.58): Comparison between thyroid parameters in newly discovered and old cases in hypothyroidism patients

Thyroid parameter	Discovery of disease	Frequency	Percent %	Mean	p.value
TSH	Old	55	49.5	5.0	0.000**
	New	56	50.5	37.1	
TT4	Old	55	49.5	82.9	0.000**
	New	56	50.5	44.5	
FT4	Old	55	49.5	14.8	0.000**
	New	56	50.5	9.4	
TT3	Old	55	49.5	1.5	0.000**
	New	56	50.5	1.2	
FT3	Old	55	49.5	3.9	0.005**
	New	56	50.5	3.3	
TPOAb	Old	55	49.5	185.3	0.693
	New	56	50.5	169.0	
TgAb	Old	55	49.5	261.2	0.126
	New	56	50.5	435.6	

***** t- test P <0.05 is significant.

Table (4.58): reviewed the correlation between discovery of disease and the means of thyroid parameters **in hypothyroidism patients**; there was highly significant correlation between **detection of disease and thyroid hormones.**

CHAPTER FIVE

Discussion
Conclusion
Recommendations

5.1 Discussion:

This study was carried out in the period of (August 2013 – May 2017) trying to bridge some informational gaps considering the evaluation of thyroid parameters and thyroid antibodies in noncancerous thyroid disease patients due to unavailability of local literature, regional and international and to find the differences and interrelationships.

The study included (183) thyroid disease patients selected by a physician according to certain criteria to fulfill the objectives of the study, then divided into two groups, group (1) hyperthyroidism patients, group (2) hypothyroidism patients and compared with (100) healthy volunteers as a control group.

The present study distributed according to thyroid disease into 111 (60.7%) with hypothyroidism, and: 72 (39.3%) with hyperthyroidism, then into sex according to disease as follows: (91.9%) of hypothyroidism were females and just (8.1%) were males, in hyperthyroidism; (84.7%) were females and (15.3%) were males

Family history: (33.3%) of **hypothyroidism** were with family history, (64.9%) of them with first degree and (35.1%) with second degree of family history and (66.7%) without a family history, **in hyperthyroidism**; (37.5%) were with positive family history (70.4%) of them with first degree and (29.6%) with second degree and (62.5%) without family history. (50.5%) of **hypothyroidism** and (41.7%) of **hyperthyroidism** patients were newly discovered.

The age in hypothyroidism; the mean of age was (50.4± 14.7 years), **in hyperthyroidism** was (43.6±13.4 years).

The body weight in hypothyroidism was (68.1±15.5 Kg), and in **hyperthyroidism** was (64.9±13.5kg).

The mean level of **TSH in hypothyroidism** was statistically significant increased than control group (*P*.value=0.000) that means there was highly significant statistical different between the means and it is out of range (0.4 – 4.3 µIU/mL), the mean **in hyperthyroidism** had statistically significant difference (*P*.value=0.000) but within reference range. There were highly significant statistically differences between the mean of thyroid parameters, in hypothyroidism, control and hyperthyroidism group (*P*.value=0.000). The results of this study were consistent with study conducted in China by **Hong Li,** *et al.,* indicating that the correlation of TT4, and fT4 with TSH was statistically significant in healthy individuals (P < 0.01). The correlation of TT4, fT4, TT3, and fT3 with TSH was statistically significant in patients with hyperthyroidism, The correlation of TT4, fT4, TT3, and fT3 with TSH was statistically significant in patients with hypothyroidism, TSH and fT4 are the most valuable indicators in assessing thyroid function in a healthy population, and TSH and TT4 are the most meaningful in hyperthyroidism and hypothyroidism. **(251)**

This present study showed that: 107 (58.5%) patients were positive when **evaluated to TPOAb** with level more than (40.0 IU/ml), 72 (64.9%) of hypothyroidism group were TPOAb positive and 35 (48.6%) of hyperthyroidism have positive titer of TPOAb, **in evaluation of TgAb**, 73 (39.9%) of the population studied were positive, 51 (69.9%) of them were hypothyroidism, and 22 (30.1%) of them were hyperthyroidism. Regarding the correlation between TPOAb and TgAb in hypothyroidism the study findings were in agreement with a study found that: the TPOAb positive patients, (60.7%) were found to be hypothyroid and TgAb positive patients;

(53.1%) patients were hypothyroid, while the correlation between thyroid antibodies and hyperthyroidism appear to be. [253] also similar results obtained from study conducted in Iran by Aminorroaya M, et al., in evaluation the prevalence of positive autoantibodies in patients with thyroid disorders, they found that; positive autoantibodies were detected in (75.5%) of patients with hypothyroidism, (73.6%) of those with hyperthyroidism. [253]

In hyperthyroidism patients; the mean level of fT3 with positive TPOAb was statistically increased more than the negative TPOAb (P.value= 0.032) that means there was statistically significant effect of presence of TPOAb on serum level of fT3.

In this instant we evaluate the correlation between clinical findings and thyroid parameters; **in hyperthyroidism patients,** there was statistically significant negative association between TPOAb, **body sweating;** (P.value=0.01). On the other hand, there was statistically positive correlation with fever, fatigue, increased appetites and fine tremor, the (P.values= 0.011, 0.027, 0.015 and 0.022) respectively. That means there was statistically significant relationship between high titer of TPOAb and these clinical findings rather than the titer of thyroid hormones.

There was a negative correlation between the TgAb and weight loss with and sweating (P.value = 0.023 and 0.04) respectively, that means the absence of these clinical findings have an association with positive titer of thyroid antibodies. While there was positive correlation with **fever, anorexia**, **exophthalmoplagia, and thick skin;** with (P.value=0.033, 0.012, 0.031 and 0.006) respectively. That means the presences of these clinical features are indicators for positive titer of TgAb.

There was statistically significant positive relationship between **fever** and **TPOAb** (p.value= 0.011),

Thyroid hormones also had significant positive correlation with some clinical findings; the clinical finding of diarrhea with fT3, with (P.value=0.036), pretibial myexodema with TT4, fT4, TT3, fT3 (P.value=0.000), (P.value = 0.044), (P.value = 0.000), (P.value = 0.008) respectively, also the tachycardia have positive correlation with TT3, (P.value = 0.046). That means the high level of thyroid hormones had statistically significant correlation with presence of these clinical findings.

This study prevailed that: That titer of thyroid hormones (TT4, fT4, TT3, and fT3) was higher in the second degree of family history than in first degree (P.value = 0.015, 0.013, 0.008, and 0.004) respectively.

In hypothyroidism patients; fT3 decreased levels had strong statistically significant correlation with restlessness, cold intolerance, with (P.value = 0.029, 0.031) respectively, while the **constipation** had statistical correlation with a decreased value in free fraction of thyroid hormones (fT4, fT3) the (P.value = 0.012, 0.005) respectively.

According to results of this research, fatigue was found to have a significant statistical correlation with TSH and thyroid hormones; (P.value = 0.030, 0.021, 0.004, 0.048) respectively.

The results findings of this present study stated that: there was a positive correlation between decrease in TT3, fT3 and proximal myopathy; (P.value= 0.046, 0.014) that explains that there was a statistically significant correlation between decrease in T3 and proximal myopathy.

Regarding a relation between **bradycardia,** there was statistically significant correlation with increase in TSH level; (P.value = 0.036).

This recent study revealed that: the clinical feature of change in voice and slow relax reflex had a positive correlation with thyroid hormones and

thyroid antibody (TSH, TT3, fT3, and TgAb); with (P.value = 0.017, 0.02, 0.034 and 0.023) respectively; that means that the change in voice and slow relax reflex had statistically positive correlation with increased TSH level and decreased total and free fraction of T3, also had positive correlation with high titer of TgAb.

The TPOAb had positive correlation with diarrhea in hypothyroidism although it is a feature of hyperthyroidism, (P.value = 0.038).

5.2 Conclusion:

- Most of thyroid patients were with hypothyroidism.
- Most thyroid dysfunctions were females.
- Free fractions of thyroid hormones have more correlations with clinical findings.
- (58.5%) of thyroid patients had TPOAb, (39.9%) had TgAb.
- Two third of hypothyroidisms had positive titer of TPOAb and TgAb
- One half of hyperthyroidisms had TPOAb positive titer and one third had TgAb
- Some clinical findings (fever, fatigue, increased appetites, fine tremor, anorexia, exophthalmoplagia, thick skin) have correlations with presence of thyroid antibodies, when the patients present with these findings, the evaluation of antibodies must be done, and the absence of clinical findings (sweating, weight loss) in hyperthyroidism indicates the presence of thyroid antibodies.

5.3 Recommendations

It is recommended that, On the basis of the obtained results of this study:

1. Evaluation of thyroid antibodies must be done for all thyroid patients
2. In thyroid hormones, free fraction recommended that total, and measurement of binding protein also important to distinguish between thyroid dysfunction and deficiency of binding proteins.
3. Some clinical findings (fever, fatigue, increased appetites, fine tremor, anorexia, exophthalmoplagia, thick skin) have correlations with presence of thyroid antibodies, when the patients present with these findings, the evaluation of antibodies must be done, and the absence of clinical findings (sweating, weight loss) in hyperthyroidism indicates the presence of thyroid antibodies.
4. Other studies must be done with large sample size and other antibodies must be included, and with other investigations e.g. serum, urine and iodine.
5. Genetic screening should be done for autoantibodies.
6. Presence of thyroid antibodies should be taken in consideration with hormonal assays to give intact results.

CHAPTER FIVE

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Appendices

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6.2 Appendices

Appendix (I)

6.2.1 Questionnaire

بسم الله الرحمن الرحيم

Assessment of Thyroid Functions and Serum Autoantibodies as Diagnostic Tools of Noncancerous Thyroid Disease Patients in Shendi Locality

No of case ☐ Age ☐

Occupation ☐ Address ☐

WT ☐

Family History ☐ Family history positive ☐

First degree ☐ Second degree ☐

- **Gender**

Male ☐ Female ☐

Symptoms and signs

Restlessness ☐ Sweating ☐

Tremor ☐ Diarrhea ☐

Constipation ☐ Fatigue ☐

Wt loss ☐ Increase appetite ☐

Hot intolerance ☐ Cold intolerance ☐

Fever ☐ Anorexia ☐

- **Eye signs**

Lid lags ☐ Lid retraction ☐

Exophthalmoses ☐ Exophthalmoplegia ☐

Loss of eye brow ☐

Proximal myopathy ☐ Pretibial myexodema ☐

Unexpressive face ☐ Thick skin ☐

Thyroid acropachy ☐ Slow relax reflex ☐

Pressure symptoms

Dysphagia ☐ Change in voice ☐

Focal thyroid sign

Solitary nodule ☐ Multinodular ☐

Diffuse ☐ No goiter ☐

Bruit ☐ No bruit ☐

Hand sign

Fine tremor ☐ Sweating ☐

Hotness ☐

Pulse

Rate ☐ Tachycardia ☐

Bradycardia ☐

Rhythm

Regular ☐ Irregular ☐

BP

Normal ☐ Hypertension ☐

Hypotension ☐

Other autoimmune disease

DM ☐ Vitiligo ☐ Addison disease ☐

Pernicious anemia ☐ SLE ☐ Rheumatoid arthritis ☐

Drug H ☐

Other systemic disorders

Pregnancy ☐ HTN Postpartum Infect ☐

Filed by:

Date

Location

Laboratory results

TSH	TT4	FT4	TT3	FT3	TPOAb	TgAb

Appendix (II)

6.2.2 Thyroid function tests

Test	Abbreviation	Normal ranges
Thyroid stimulating hormone	TSH	0.5–6.0 µU/ml
Free thyroxine	FT_4	7–18 ng/l = 0.7–1.8 ng/dl
Serum triiodothyronine	T_3	0.8–1.8 µg/l = 80-180 ng/dl
Radioactive iodine-123 uptake	RAIU	10–30%
Radioiodine scan (gamma camera)		N/A thyroid contrasted images
Free thyroxine fraction	FT_4F	0.03–0.005%
Serum thyroxine	T_4	46–120 µg/l = 4.6–12.0 µg/dl
Thyroid hormone binding ratio	THBR	0.9–1.1
Free thyroxine index	FT_4I	4–11
Free triiodothyronine l	FT_3	230–619 pg/d
Free T3 Index	FT_3I	80–180
Thyroxine-binding globulin	TBG	12–20 ug/dl T4 +1.8 µg
TRH stimulation test	Peak TSH	9–30 µIU/ml at 20–30 min.
Serum thyroglobulin l	Tg	0-30 ng/m
Thyroid microsomal antibody titer	TMAb	Varies with method
Thyroglobulin antibody titer	TgAb	Varies with method

Appendix (III)

6.2.3 Effects of some drugs on Tests of Thyroid function [252]

Drug	Cause	Effect
Dopamine, L-dopa, Glucocorticoids, Somatostatin	Inhibit TSH secretion	$\downarrow T_4$; $\downarrow T_3$; $\downarrow$TSH
Iodine, Lithium	Inhibit thyroid hormone synthesis or release	$\downarrow T_4$; $\downarrow T_3$; $\uparrow$TSH
Amiodarone, Glucocorticoids, Propranolol, Propylthiouracil, Radiographic contrast agents	Inhibit conversion of T_4 to T_3	$\downarrow T_3$; $\uparrow rT_3$; $\downarrow$, $\leftrightarrow$, $\uparrow T_4$ and fT_4; $\leftrightarrow$, $\uparrow$TSH
Salicylates, Phenytoin, Carbamazepine, Furosemide, Nonsteroidal anti-inflammatory agents, Heparin (in vitro effect)	Inhibit binding of T_4/T_3 to serum proteins	$\downarrow T_4$;$\downarrow T_3$; $\downarrow fT_4E$, $\leftrightarrow$,$\uparrow fT_4$; $\leftrightarrow$TSH
Phenobarbital, Phenytoin, Carbamazepine, Rifampicin	Stimulate metabolism of iodothyronines	$\downarrow T_4$;$\downarrow fT_4$;$\leftrightarrow$TSH
Aluminium hydroxide, Ferrous sulfate, Cholestyramine, Colestipol, Iron sucralfate, Soybean preparations, Kayexalate	Inhibit absorption of ingested T_4	$\downarrow T_4$; $\downarrow fT_4$; $\uparrow$TSH
Estrogen, Clofibrate, Opiates (heroin, methadone), 5-Fluorouracil, Perphenzazine	Increase in concentration of T_4-binding proteins	$\uparrow T_4$; $\uparrow T_3$; $\leftrightarrow fT_4$; $\leftrightarrow$TSH
Androgens, Glucocorticoids	Decrease in concentration of T4-binding proteins	$\downarrow T_4$; $\downarrow T_3$; $\leftrightarrow fT_4$; $\leftrightarrow$TSH

Appendix (IV)

6.2.4 Recommended levothyroxine (L-T4) treatment doses

Age	Dose (mcg/kg/day
0–3 months	10–12
3–6 months	8–10
6–12 months	6–8
1–3 years	4–6
3–10 years	3–4
10–15 years	2–4
>15 years	2–3
Adult	1.6–1.8

Appendix (V)

6.2.5 Reagent preparation:

- **Substrate solution**

Bring all reagents to (18-25 ºC) before preparing the working reagent; add the entire content of the AIA – PACK SUBSTRATE RECONSTITUENT II (100mL) to the lyophilized AIA – PACK SUBSTRATE REAGENT II and mix thoroughly to dissolve the solid material.

- **Wash solution**

Add the entire contents of the AIA – PACK wash concentrate (100mL) to approximately (2.0 L) of class 1 water or the clinical laboratory reagent water, mix well, and adjust the final volume to (2.5 L).

- **Diluent**

Add the entire contents of the AIA – PACK diluent concentrate (100 mL) to approximately (4.0 L) of class 1 water or the clinical laboratory reagent water mix well, and adjust the final volume to (5.0 L).

Appendix (VI)

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Attention

For North and South American Customers: Please refer to the AIA-AAM Docs on CD for the appropriate information.

Para los Clientes en Norte y Sur América: favor de referirse a los documentos AIA-AAM en Disco para la información apropiada.

Aos clientes da América do Norte e América do Sul: favor consultar os documentos do AIA-AAM que estão em CD para informações adequadas.

Pour les clients en Amérique du Nord et en Amérique du Sud: veuillez consulter les documents AIA-AAM sur le CD pour l' information appropriée.

ST AIA-PACK TSH

For Quantitative Measurement of thyroid stimulating hormone (TSH or thyrotropin) in Serum or Heparinized Plasma

NAME AND INTENDED USE

ST AIA-PACK TSH is designed for IN VITRO DIAGNOSTIC USE ONLY for the quantitative measurement of thyroid stimulating hormone (TSH or thyrotropin) in human serum or heparinized plasma on TOSOH AIA System Analyzers.

SUMMARY AND EXPLANATION OF TEST

Thyroid stimulating hormone is a glycoprotein hormone secreted by the anterior pituitary gland. When feedback suppression of the pituitary is reduced by a reduced production of thyroid hormones (T_4 and T_3), TSH rises in an attempt to increase thyroid hormone production. This rise occurs while the patient is still asymptomatic and thus is an early and very sensitive indication of hypothyroidism *(1-5)*. TSH is also controlled by the hypothalamic peptide, thyrotropin releasing hormone (TRH).

Accurate determination of serum TSH is the most useful and sensitive test for primary hypothyroidism, where serum thyroid hormone concentrations are depressed and serum TSH concentrations are significantly elevated. Serum TSH determinations may also be used to differentiate between pituitary (secondary) and hypothalamic (tertiary) hypothyroidisms *(6-9)*. Through the use of monoclonal antibody technology which provides the necessary specificity and sensitivity, the usefulness of TSH determination in the diagnosis of hyperthyroidism distinguished from euthyroidism has been well established *(10,11)*.

PRINCIPLE OF THE ASSAY

The ST AIA-PACK TSH is a two-site immunoenzymometric assay which is performed entirely in the ST AIA-PACK TSH test cups. TSH present in the test sample is bound with monoclonal antibody immobilized on magnetic beads and monoclonal antibody conjugated with bovine alkaline phosphatase in the test cups. The magnetic beads are washed to remove unbound enzyme-labeled monoclonal antibody and are then incubated with a fluorogenic substrate, 4-methylumbelliferyl phosphate (4MUP). The amount of enzyme conjugated with monoclonal antibody that binds to the beads is directly proportional to the TSH concentration in the test sample. A standard curve is constructed, and unknown sample concentrations are calculated using this curve.

MATERIAL PROVIDED (ST AIA-PACK TSH, Cat. No. 0025294)

5 trays x 20 test cups

Plastic test cups containing lyophilized twelve magnetic beads coated with anti-TSH mouse monoclonal antibody and 50 µL of anti-TSH mouse monoclonal antibody conjugated to bovine alkaline phosphatase with sodium azide as a preservative.

MATERIALS REQUIRED BUT NOT PROVIDED

The following materials are required to perform TSH analysis using the ST AIA-PACK TSH (Cat. No. 0025294) on the TOSOH AIA System Analyzers. They are available separately from TOSOH.

Materials			Cat. No.
AIA Nex-IA or AIA-21			0018539
AIA Nex-IA or AIA-21 LA			0018540
AIA-1800 ST			0019836
AIA-1800 LA			0019837
AIA-2000 ST			0022100
AIA-2000 LA			0022101
AIA-600 II			0019014
AIA-600 II BCR			0019328
AIA-900			0022930
AIA-360			0019945
AIA-PACK SUBSTRATE SET II AIA-PACK SUBSTRATE REAGENT II AIA-PACK SUBSTRATE RECONSTITUENT II			0020968
AIA-PACK TSH 3rd-Gen CALIBRATOR SET			0020394
AIA-PACK TSH 3rd-Gen CALIBRATOR (1)	0	µIU/mL	
AIA-PACK TSH 3rd-Gen CALIBRATOR (2)	0.2	µIU/mL (approx.)	
AIA-PACK TSH 3rd-Gen CALIBRATOR (3)	5.0	µIU/mL (approx.)	
AIA-PACK TSH 3rd-Gen CALIBRATOR (4)	25	µIU/mL (approx.)	
AIA-PACK TSH 3rd-Gen CALIBRATOR (5)	50	µIU/mL (approx.)	
AIA-PACK TSH 3rd-Gen CALIBRATOR (6)	110	µIU/mL (approx.)	
AIA-PACK TSH 3rd-Gen SAMPLE DILUTING SOLUTION			0020594
AIA-PACK WASH CONCENTRATE			0020955
AIA-PACK DILUENT CONCENTRATE			0020956
SAMPLE CUPS			0018581
AIA-PACK DETECTOR STANDARDIZATION TEST CUP			0020970
AIA-PACK SAMPLE TREATMENT CUP			0020971
Additional Requirements for AIA Nex-IA / AIA-21 only:			
PIPETTE TIPS			0018552
PRELOADED PIPETTE TIPS			0018583
Additional Requirements for AIA-600 II, AIA-900, AIA-1800 and AIA-2000:			
PIPETTE TIPS			0019215
TIP RACK			0019216
PRELOADED PIPETTE TIPS			0022103

Only materials obtained from TOSOH should be used. Materials obtained elsewhere should not be substituted since assay performance is characterized based strictly on TOSOH materials.

WARNINGS AND PRECAUTIONS

1. The ST AIA-PACK TSH is intended for in vitro diagnostic use only.
2. Inspect the packaging and the exterior of the aluminum pouch for any sign of damage before use. If any damages are visible, contact your local TOSOH sales representative.
3. Test cups from different lots or different assays shall not be mixed within a tray.
4. The ST AIA-PACK TSH contains sodium azide, which may react with lead or copper plumbing to form potentially explosive metal azides. When disposing of such reagents, always flush with large volumes of water to prevent azide build-up.
5. Human serum is not used in the preparation of this product; however, since human specimens will be used for samples and other quality control products in the lab may be derived from human serum, please use standard laboratory safety procedures in handling all specimens and controls.
6. Do not use beyond the expiration date.
7. The ST AIA-PACK TSH has been designed so that the high dose "hook effect" is not a problem for the vast majority of samples. Samples with TSH concentrations between 100 and 5,000 µIU/mL will read >100 µIU/mL. The "hook effect" phenomenon may occur at TSH concentrations >5,000 µIU/mL.
8. For safe waste disposal, it is recommended that each laboratory complies with established laboratory procedures and local, state, and federal regulations.
9. After opening, the vial of AIA-PACK TSH 3rd-Gen SAMPLE DILUTING SOLUTION should be kept tightly sealed with a clean rubber cap. Sealing with dirty material may cause deterioration of the reagent.
10. The remaining sample diluting solution after use should not be mixed with another vial but be discarded to avoid contamination.
11. Serum, dust, metal, or microorganism contamination may cause degradation of reconstituted substrate solution. Store in a clean environment, away from direct sunlight and ultraviolet light.
12. TOSOH recommends that a new pouch of the test cups should be used for calibration.

STORAGE AND STABILITY

All unopened materials are stable until the expiration date on the label when stored at the specified temperature.

Materials	Cat. No.
2-8°C:	
ST AIA-PACK TSH	0025294
AIA-PACK TSH 3rd-Gen CALIBRATOR SET	0020394
AIA-PACK TSH 3rd-Gen SAMPLE DILUTING SOLUTION	0020594
AIA-PACK SUBSTRATE SET II	0020968
AIA-PACK WASH CONCENTRATE	0020955
AIA-PACK DILUENT CONCENTRATE	0020956
1-30°C:	
AIA-PACK DETECTOR STANDARDIZATION TEST CUP	0020970
AIA-PACK SAMPLE TREATMENT CUP	0020971

After opening the aluminum pouch, ST AIA-PACK TSH test cups can be left on-board of the TOSOH AIA System Analyzers (18-25°C) for a maximum of 10 days (10 x 24 hours). When stored over night at 2-8°C, the test cups can be used for up to 30 days (30 cycles of 8 hours on board and 16 hours in the refrigerator). Once the aluminum pouch is opened, the test cups must be used within 30 days.

AIA-PACK TSH 3rd-Gen CALIBRATOR SET must be kept tightly sealed and refrigerated at 2-8°C. After opening, the calibrators should be used within 1 day.

After opening, AIA-PACK TSH 3rd-Gen SAMPLE DILUTING SOLUTION can be left on-board of the TOSOH AIA System Analyzers (18-25°C) for a maximum of 3 days (3 x 24 hours). When stored over night at 2-8°C, the sample diluting solution can be used for up to 9 days (9 cycles of 8 hours on board and 16 hours in the refrigerator). The sample diluting solution should not be used beyond 90 days after opening, even if it is sealed and stored in the refrigerator.

Reconstituted substrate solution is stable for 3 days at 18-25°C or 30 days at 2-8°C. Working diluent and wash solutions are stable for 30 days at 18-25°C.

Reagents should not be used if they appear cloudy or discolored.

SPECIMEN COLLECTION AND HANDLING

1. Serum or heparinized plasma is required for the assay. EDTA and citrated plasma SHOULD NOT BE USED.
2. When using serum, a venous blood sample is collected aseptically without additives. Store at 18-25°C until a clot has formed (usually 15-45 minutes), then centrifuge to obtain the serum specimen for assay.
3. When using heparinized plasma, a venous blood sample is collected aseptically with designated additive. Centrifuge and separate plasma from the packed cells as soon as possible.
4. Inadequate centrifugation or the presence of fibrin or particulate matter in the sample may cause an erroneous result.
5. Samples containing inhibitors of alkaline phosphatase may cause erroneous results.
6. Inspect all samples for air bubbles and foaming. Remove any air bubbles prior to assay.
7. Specimen types should not be used interchangeably during serial monitoring of an individual patient. Measured concentrations may vary slightly between sample types in certain patients.
8. Samples may be stored at 2-8°C for up to 7 days prior to analysis. If the analysis cannot be done within 7 days, the sample should be stored frozen at -20°C or below for up to 60 days.
9. Repeated freeze-thaw cycles should be avoided. Turbid serum samples or samples containing particulate matter should be centrifuged prior to testing. Prior to assay, bring frozen samples to 18-25°C slowly and mix gently.
10. The sample required for analysis is 100 µL.

PROCEDURE

For the AIA Nex-IA / AIA-21, AIA-600 II, AIA-900, AIA-1800, AIA-2000 and AIA-360, please refer to their Operator's Manual for detailed instructions.

I. Reagent Preparation

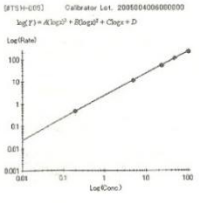
A) Substrate Solution
Bring all reagents to 18-25°C before preparing the working reagent. Add the entire contents of the AIA-PACK SUBSTRATE RECONSTITUENT II (100 mL) to the lyophilized AIA-PACK SUBSTRATE REAGENT II and mix thoroughly to dissolve the solid material.

B) Wash Solution
Add the entire contents of the AIA-PACK WASH CONCENTRATE (100 mL) to approximately 2.0 L of CAP Class I water or the clinical laboratory reagent water (formerly NCCLS Type I) defined by CLSI C3-A4 guideline, mix well, and adjust the final volume to 2.5 L.

C) Diluent
Add the entire contents of the AIA-PACK DILUENT CONCENTRATE (100 mL) to approximately 4.0 L of CAP Class I water or the clinical laboratory reagent water (formerly NCCLS Type I) defined by CLSI C3-A4 guideline, mix well, and adjust the final volume to 5.0 L.

II. Calibration Procedure

A) Calibration Curve
The calibrators for use with the ST AIA-PACK TSH have been standardized on WHO 2nd IRP 80/558 (1983).
The calibration curve for ST AIA-PACK TSH is stable for up to 90 days. Calibration stability is monitored by quality control performance and is dependent on proper reagent handling and TOSOH AIA System maintenance according to the manufacturer's instructions.
Recalibration may be necessary more frequently if controls are out of the established range for this assay or when certain service procedures are performed (e.g. temperature adjustment, sampling mechanism changes, maintenance of the wash probe, or detector lamp adjustment or change). For further information regarding instrument operation, consult the TOSOH AIA System Operator's Manual.
A sample calibration curve from AIA-1800 follows and shows the algorithm used for calculating results.



Appendix (VII)

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Rev. 07/12

Attention

For North and South American Customers: Please refer to the AIA-AAM Docs on CD for the appropriate information.

Para los Clientes en Norte y Sur América: favor de referirse a los documentos AIA-AAM en Disco para la información apropiada.

Aos clientes da América do Norte e América do Sul: favor consultar os documentos do AIA-AAM que estão em CD para informações adequadas.

Pour les clients en Amérique du Nord et en Amérique du Sud: veuillez consulter les documents AIA-AAM sur le CD pour l' information appropriée.

ST AIA-PACK T4

For Quantitative Measurement of thyroxine (T_4) in Serum or Heparinized Plasma

NAME AND INTENDED USE

ST AIA-PACK T4 is designed for IN VITRO DIAGNOSTIC USE ONLY for the quantitative measurement of thyroxine (T_4) in human serum or heparinized plasma on TOSOH AIA System Analyzers.

SUMMARY AND EXPLANATION OF TEST

Evaluation of thyroid status is complex. The primary function of the thyroid gland is the secretion of thyroxine (T_4) or triiodothyronine (T_3). Abnormal secretion of T_4 and/or T_3 may lead either to hyper- or hypo-thyroidism. The synthesis and release of T_4 and T_3 are in response to a hypothalamic-pituitary signal, thyroid stimulating hormone (TSH), which is released from the anterior pituitary and is the principal regulator of thyroid activity *(1)*. The release of TSH is controlled by thyrotropin releasing hormone (TRH) from the hypothalamus *(2)*. This combined system regulating the release of thyroid hormone is the hypothalamic-pituitary axis *(3, 4)*. In the circulation, T_4 is 99.97% protein bound (0.03% free) while T_3 is 99.7% bound (0.3% free). Thyroxine binding globulin (TBG) is the primary binding protein. To a lesser extent, thyroxine binding prealbumin (TBPA) and albumin can also bind T_4 *(5, 6, 7)*. Only unbound (free) forms exert the physiological actions.

T_4 is largely converted to T_3 in peripheral tissues by monodeiodinatio *(8)*. Total T_4 rises and falls with the TBG level in euthyroid individuals. An erroneous interpretation of thyroid function may be obtained if a condition which changes the TBG concentrations exists. Certain drugs compete with T_4 for binding to TBG, which results in decreased levels of total T_4 through the negative feedback of thyroid hormone concentration on TSH secretion *(9, 10)*.

PRINCIPLE OF THE ASSAY

The ST AIA-PACK T4 is a competitive enzyme immunoassay which is performed entirely in the ST AIA-PACK T4 test cups. Thyroxine, which is displaced from its binding proteins by ANS (8-anilino-1-naphthalene sulfonic acid), and free T_4 present in the test sample compete with enzyme-labeled thyroxine for a limited number of binding sites on a thyroxine-specific antibody immobilized on magnetic beads. The beads are washed to remove the unbound enzyme-labeled thyroxine and are then incubated with a fluorogenic substrate, 4-methylumbelliferyl phosphate (4MUP). The amount of enzyme-labeled thyroxine that binds to the beads is inversely proportional to the thyroxine concentration in the test sample. A standard curve using a range of known standard concentrations is constructed and unknown thyroxine concentrations are calculated using this curve.

MATERIAL PROVIDED (ST AIA-PACK T4, Cat. No. 0025258)

5 trays x 20 test cups

Plastic test cups containing lyophilized twelve magnetic beads with anti-T_4 rabbit polyclonal antibody, 140 µL of T_4 conjugated to bovine alkaline phosphatase and ANS (8-anilino-1-naphthalene sulfonic acid) with sodium azide as a preservative.

MATERIALS REQUIRED BUT NOT PROVIDED

The following materials are required to perform thyroxine analysis using the ST AIA-PACK T4 (Cat. No. 0025258) on the TOSOH AIA Systems Analyzers. They are available separately from TOSOH.

Material			Cat. No.
AIA Nex-IA or AIA-21			0018539
AIA Nex-IA or AIA-21 LA			0018540
AIA-1800 ST			0019836
AIA-1800 LA			0019837
AIA-2000 ST			0022100
AIA-2000 LA			0022101
AIA-600 II			0019014
AIA-600 II BCR			0019328
AIA-900			0022930
AIA-360			0019945
AIA-PACK SUBSTRATE SET II			0020968
AIA-PACK SUBSTRATE REAGENT II			
AIA-PACK SUBSTRATE RECONSTITUENT II			
AIA-PACK T4 CALIBRATOR SET			0020358
AIA-PACK T4 CALIBRATOR (1)	0	µg/dL	
AIA-PACK T4 CALIBRATOR (2)	0.75	µg/dL (approx.)	
AIA-PACK T4 CALIBRATOR (3)	3.0	µg/dL (approx.)	
AIA-PACK T4 CALIBRATOR (4)	6.0	µg/dL (approx.)	
AIA-PACK T4 CALIBRATOR (5)	12	µg/dL (approx.)	
AIA-PACK T4 CALIBRATOR (6)	26	µg/dL (approx.)	
AIA-PACK T4 SAMPLE DILUTING SOLUTION			0020558
AIA-PACK WASH CONCENTRATE			0020955
AIA-PACK DILUENT CONCENTRATE			0020956
SAMPLE CUPS			0018581
AIA-PACK DETECTOR STANDARDIZATION TEST CUP			0020970
AIA-PACK SAMPLE TREATMENT CUP			0020971
Additional Requirements for AIA Nex-IA / AIA-21 only:			
PIPETTE TIPS			0018552
PRELOADED PIPETTE TIPS			0018583
Additional Requirements for AIA-600 II, AIA-900, AIA-1800 and AIA-2000:			
PIPETTE TIPS			0019215
TIP RACK			0019216
PRELOADED PIPETTE TIPS			0022103

Only materials obtained from TOSOH should be used. Materials obtained elsewhere should not be substituted since assay performance is characterized based strictly on TOSOH materials.

WARNINGS AND PRECAUTIONS

1. The ST AIA-PACK T4 is intended for in vitro diagnostic use only.
2. Inspect the packaging and the exterior of the aluminum pouch for any sign of damage before use. If any damages are visible, contact your local TOSOH sales representative.
3. Test cups from different lots or different assays shall not be mixed within a tray.
4. The ST AIA-PACK T4 contains sodium azide, which may react with lead or copper plumbing to form potentially explosive metal azides. When disposing of such reagents, always flush with large volumes of water to prevent azide build-up.
5. Human serum is not used in the preparation of this product; however, since human specimens will be used for samples and other quality control products in the lab may be derived from human serum, please use standard laboratory safety procedures in handling all specimens and controls.
6. Do not use beyond the expiration date.
7. For safe waste disposal, it is recommended that each laboratory complies with established laboratory procedures and local, state, and federal regulations.
8. After opening, the vial of AIA-PACK T4 SAMPLE DILUTING SOLUTION should be kept tightly sealed with a clean rubber cap. Sealing with dirty material may cause deterioration of the reagent.
9. The remaining sample diluting solution after use should not be mixed with another vial but be discarded to avoid contamination.
10. Serum, dust, metal, or microorganism contamination may cause degradation of reconstituted substrate solution. Store in a clean environment, away from direct sunlight and ultraviolet light.
11. TOSOH recommends that a new pouch of the test cups should be used for calibration.

STORAGE AND STABILITY

All unopened materials are stable until the expiration date on the label when stored at the specified temperature.

Materials	Cat. No.
2-8°C:	
ST AIA-PACK T4	0025258
AIA-PACK T4 CALIBRATOR SET	0020358
AIA-PACK T4 SAMPLE DILUTING SOLUTION	0020558
AIA-PACK SUBSTRATE SET II	0020968
AIA-PACK WASH CONCENTRATE	0020955
AIA-PACK DILUENT CONCENTRATE	0020956
1-30°C:	
AIA-PACK DETECTOR STANDARDIZATION TEST CUP	0020970
AIA-PACK SAMPLE TREATMENT CUP	0020971

After opening the aluminum pouch, ST AIA-PACK T4 test cups can be left on-board of the TOSOH AIA System Analyzers (18-25°C) for a maximum of 10 days (10 x 24 hours). When stored over night at 2-8°C, the test cups can be used for up to 30 days (30 cycles of 8 hours on board and 16 hours in the refrigerator). Once the aluminum pouch is opened, the test cups must be used within 30 days.

AIA-PACK T4 CALIBRATOR SET must be kept tightly sealed and refrigerated at 2-8°C. After opening, the calibrators should be used within 1 day.

After opening, AIA-PACK T4 SAMPLE DILUTING SOLUTION can be left on-board of the TOSOH AIA System Analyzers (18-25°C) for a maximum of 3 days (3 x 24 hours). When stored over night at 2-8°C, the sample diluting solution can be used for up to 9 days (9 cycles of 8 hours on board and 16 hours in the refrigerator). The sample diluting solution should not be used beyond 90 days after opening, even if it is sealed and stored in the refrigerator.

Reconstituted substrate solution is stable for 3 days at 18-25°C or 30 days at 2-8°C. Working diluent and wash solutions are stable for 30 days at 18-25°C.

Reagents should not be used if they appear cloudy or discolored.

SPECIMEN COLLECTION AND HANDLING

1. Serum or heparinized plasma is required for the assay. EDTA and citrated plasma SHOULD NOT BE USED.
2. When using serum, a venous blood sample is collected aseptically without additives. Store at 18-25°C until a clot has formed (usually 15-45 minutes), then centrifuge to obtain the serum specimen for assay.
3. When using heparinized plasma, a venous blood sample is collected aseptically with designated additive. Centrifuge and separate plasma from the packed cells as soon as possible.
4. Inadequate centrifugation or the presence of fibrin or particulate matter in the sample may cause an erroneous result.
5. Samples containing inhibitors of alkaline phosphatase may cause erroneous results.
6. Inspect all samples for air bubbles and foaming. Remove any air bubbles prior to assay.
7. Specimen types should not be used interchangeably during serial monitoring of an individual patient. Measured concentrations may vary slightly between sample types in certain patients.
8. Samples may be stored at 2-8°C for up to 24 hours prior to analysis. If the analysis cannot be done within 24 hours, the sample should be stored frozen at –20°C or below for up to 60 days.
9. Repeated freeze-thaw cycles should be avoided. Turbid serum samples or samples containing particulate matter should be centrifuged prior to testing. Prior to assay, bring frozen samples to 18-25°C slowly and mix gently.
10. The sample required for analysis is 10 µL.

PROCEDURE

For the AIA Nex-IA / AIA-21, AIA-600 II, AIA-900, AIA-1800, AIA-2000 and AIA-360, please refer to their Operator's Manual for detailed instructions.

I. Reagent Preparation

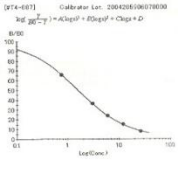
A) Substrate Solution
Bring all reagents to 18-25°C before preparing the working reagent. Add the entire contents of the AIA-PACK SUBSTRATE RECONSTITUENT II (100 mL) to the lyophilized AIA-PACK SUBSTRATE REAGENT II and mix thoroughly to dissolve the solid material.

B) Wash Solution
Add the entire contents of the AIA-PACK WASH CONCENTRATE (100 mL) to approximately 2.0 L of CAP Class I water or the clinical laboratory reagent water (formerly NCCLS Type I) defined by CLSI C3-A4 guideline, mix well, and adjust the final volume to 2.5 L.

C) Diluent
Add the entire contents of the AIA-PACK DILUENT CONCENTRATE (100 mL) to approximately 4.0 L of CAP Class I water or the clinical laboratory reagent water (formerly NCCLS Type I) defined by CLSI C3-A4 guideline, mix well, and adjust the final volume to 5.0 L.

II. Calibration Procedure

A) Calibration Curve
The calibrators for use with the ST AIA-PACK T4 are prepared gravimetrically and are compared to internal reference standards.
The calibration curve for ST AIA-PACK T4 is stable for up to 90 days. Calibration stability is monitored by quality control performance and is dependent on proper reagent handling and TOSOH AIA System maintenance according to the manufacturer's instructions.
Recalibration may be necessary more frequently if controls are out of the established range for this assay or when certain service procedures are performed (e.g. temperature adjustment, sampling mechanism changes, maintenance of the wash probe, or detector lamp adjustment or change). For further information regarding instrument operation, consult the TOSOH AIA System Operator's Manual.
A sample calibration curve from AIA-1800 follows and shows the algorithm used for calculating results.



Appendix (VIII)

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Rev. 07/12

Attention

For North and South American Customers: Please refer to the AIA-AAM Docs on CD for the appropriate information.

Para los Clientes en Norte y Sur América: favor de referirse a los documentos AIA-AAM en Disco para la información apropiada.

Aos clientes da América do Norte e América do Sul: favor consultar os documentos do AIA-AAM que estão em CD para informações adequadas.

Pour les clients en Amérique du Nord et en Amérique du Sud: veuillez consulter les documents AIA-AAM sur le CD pour l' information appropriée.

ST AIA-PACK FT4

For Quantitative Measurement of non-protein-bound (free) thyroxine (FT_4) in Serum or Heparinized Plasma

NAME AND INTENDED USE

ST AIA-PACK FT4 is designed for IN VITRO DIAGNOSTIC USE ONLY for the quantitative measurement of non-protein-bound (free) thyroxine (FT_4) in human serum or heparinized plasma on TOSOH AIA System Analyzers.

SUMMARY AND EXPLANATION OF TEST

L-thyroxine (3, 5, 3-, 5-L-tetraiodothyronine (T_4)) produced by the thyroid gland, circulates in the blood 99.97% bound to plasma proteins including thyroxine-binding globulin (TBG), thyroxine-binding prealbumin (TBPA) and albumin *(1,2)*. Approximately 0.03% of the total circulating thyroxine is unbound. This free T_4 (FT_4) is believed to be the physiologically active portion of the thyroxine which stimulates the metabolism and controls, via the pituitary, the feedback system involving the release of TSH *(3)*. Historically, measurement of total serum T_4 (bound + free) has been used to assess the clinical status of the thyroid gland *(4,5)*. However, this analysis is not diagnostically accurate when significant changes occur in the serum binding proteins *(6,7)*. Alterations in TBG concentration, pregnancy, oral contraceptives, estrogen therapy or drugs which alter the binding of thyroxine to the carrier proteins may cause corresponding changes in the total T_4 when unbound free thyroxine levels remain relatively unchanged *(8,9)*. Therefore, measurement of free T_4 (FT_4) typically correlates more closely to the patient's actual thyroid status than the total *(10)*.

PRINCIPLE OF THE ASSAY

The ST AIA-PACK FT4 is a competitive enzyme immunoassay which is performed entirely in the ST AIA-PACK FT4 test cups. The thyroxine not bound to serum proteins (free T_4) competes with enzyme-labeled T_4 for a limited number of binding sites on a T_4-specific antibody immobilized on magnetic beads. After incubation, the beads are washed to remove the unbound enzyme-labeled T_4 and are then incubated with a fluorogenic substrate, 4-methylumbelliferyl phosphate (4MUP). The amount of enzyme-labeled T_4 that binds to the beads is inversely proportional to the free T_4 concentration in the test sample. A standard curve using a range of known standard concentrations is constructed and unknown sample free T_4 concentrations are calculated using this curve.

MATERIAL PROVIDED (ST AIA-PACK FT4, Cat. No. 0025268)

5 trays x 20 test cups

Plastic test cups containing lyophilized twelve magnetic beads with anti-T_4 rabbit polyclonal antibody and 140 μL of thyroxine (T_4) conjugated to bovine alkaline phosphatase with sodium azide as a preservative.

MATERIALS REQUIRED BUT NOT PROVIDED

The following materials are required to perform free thyroxine analysis using the ST AIA-PACK FT4 (Cat. No. 0025268) on the TOSOH AIA System Analyzers. They are available separately from TOSOH.

Materials			Cat. No.
AIA Nex-IA or AIA-21			0018539
AIA Nex-IA or AIA-21 LA			0018540
AIA-1800 ST			0019836
AIA-1800 LA			0019837
AIA-2000 ST			0022100
AIA-2000 LA			0022101
AIA-600 II			0019014
AIA-600 II BCR			0019328
AIA-900			0022930
AIA-360			0019945
AIA-PACK SUBSTRATE SET II			0020968
AIA-PACK SUBSTRATE REAGENT II			
AIA-PACK SUBSTRATE RECONSTITUENT II			
AIA-PACK FT4 CALIBRATOR SET			0020368
AIA-PACK FT4 CALIBRATOR (1)	0	ng/dL	
AIA-PACK FT4 CALIBRATOR (2)	0.4	ng/dL (approx.)	
AIA-PACK FT4 CALIBRATOR (3)	1.0	ng/dL (approx.)	
AIA-PACK FT4 CALIBRATOR (4)	2.0	ng/dL (approx.)	
AIA-PACK FT4 CALIBRATOR (5)	4.0	ng/dL (approx.)	
AIA-PACK FT4 CALIBRATOR (6)	9.0	ng/dL (approx.)	
AIA-PACK WASH CONCENTRATE			0020955
AIA-PACK DILUENT CONCENTRATE			0020956
SAMPLE CUPS			0018581
AIA-PACK DETECTOR STANDARDIZATION TEST CUP			0020970

Additional Requirements for AIA Nex-IA / AIA-21 only:

PIPETTE TIPS	0018552
PRELOADED PIPETTE TIPS	0018583

Additional Requirements for AIA-600 II, AIA-900, AIA-1800 and AIA-2000:

PIPETTE TIPS	0019215
TIP RACK	0019216
PRELOADED PIPETTE TIPS	0022103

Only materials obtained from TOSOH should be used. Materials obtained elsewhere should not be substituted since assay performance is characterized based strictly on TOSOH materials.

WARNINGS AND PRECAUTIONS

1. The ST AIA-PACK FT4 is intended for in vitro diagnostic use only.
2. Inspect the packaging and the exterior of the aluminum pouch for any sign of damage before use. If any damages are visible, contact your local TOSOH sales representative.
3. Test cups from different lots or different assays shall not be mixed within a tray.
4. The ST AIA-PACK FT4 contains sodium azide, which may react with lead or copper plumbing to form potentially explosive metal azides. When disposing of such reagents, always flush with large volumes of water to prevent azide build-up.
5. Human serum is not used in the preparation of this product; however, since human specimens will be used for samples and other quality control products in the lab may be derived from human serum, please use standard laboratory safety procedures in handling all specimens and controls.
6. Do not use beyond the expiration date.
7. For safe waste disposal, it is recommended that each laboratory complies with established laboratory procedures and local, state, and federal regulations.
8. Serum, dust, metal, or microorganism contamination may cause degradation of reconstituted substrate solution. Store in a clean environment, away from direct sunlight and ultraviolet light.
9. TOSOH recommends that a new pouch of the test cups should be used for calibration.

STORAGE AND STABILITY

All unopened materials are stable until the expiration date on the label when stored at the specified temperature.

Materials	Cat. No.
2-8°C:	
ST AIA-PACK FT4	0025268
AIA-PACK FT4 CALIBRATOR SET	0020368
AIA-PACK SUBSTRATE SET II	0020968
AIA-PACK WASH CONCENTRATE	0020955
AIA-PACK DILUENT CONCENTRATE	0020956
1-30°C:	
AIA-PACK DETECTOR STANDARDIZATION TEST CUP	0020970

After opening the aluminum pouch, ST AIA-PACK FT4 test cups can be left on-board of the TOSOH AIA System Analyzers (18-25°C) for a maximum of 1 day (24 hours). When stored over night at 2-8°C, the test cups can be used for up to 3 days (3 cycles of 8 hours on board and 16 hours in the refrigerator). Once the aluminum pouch is opened, even if the test cups are stored in the refrigerator, they must be used within 30 days.
AIA-PACK FT4 CALIBRATOR SET must be kept tightly sealed and refrigerated at 2-8°C. After opening or reconstituting, the calibrators should be used within 1 day.
Reconstituted substrate solution is stable for 3 days at 18-25°C or 30 days at 2-8°C. Working diluent and wash solutions are stable for 30 days at 18-25°C.
Reagents should not be used if they appear cloudy or discolored.

SPECIMEN COLLECTION AND HANDLING

1. Serum or heparinized plasma is required for the assay. EDTA and citrated plasma SHOULD NOT BE USED.
2. When using serum, a venous blood sample is collected aseptically without additives. Store at 18-25°C until a clot has formed (usually 15-45 minutes), then centrifuge to obtain the serum specimen for assay.
3. When using heparinized plasma, a venous blood sample is collected aseptically with designated additive. Centrifuge and separate plasma from the packed cells as soon as possible.
4. Inadequate centrifugation or the presence of fibrin or particulate matter in the sample may cause an erroneous result.
5. Samples containing inhibitors of alkaline phosphatase may cause erroneous results.
6. Inspect all samples for air bubbles and foaming. Remove any air bubbles prior to assay.
7. Specimen types should not be used interchangeably during serial monitoring of an individual patient. Measured concentrations may vary slightly between sample types in certain patients.
8. Samples may be stored at 2-8°C for up to 24 hours prior to analysis. If the analysis cannot be done within 24 hours, the sample should be stored frozen at –20°C or below for up to 60 days.
9. Repeated freeze-thaw cycles should be avoided. Turbid serum samples or samples containing particulate matter should be centrifuged prior to testing. Prior to assay, bring frozen samples to 18-25°C slowly and mix gently.
10. The sample required for analysis is 10 μL.

PROCEDURE

For the AIA Nex-IA / AIA-21, AIA-600 II, AIA-900, AIA-1800, AIA-2000 and AIA-360, please refer to their Operator's Manual for detailed instructions.

I. Reagent Preparation

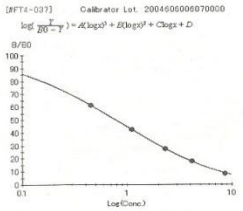
A) Substrate Solution
Bring all reagents to 18-25°C before preparing the working reagent. Add the entire contents of the AIA-PACK SUBSTRATE RECONSTITUENT II (100 mL) to the lyophilized AIA-PACK SUBSTRATE REAGENT II and mix thoroughly to dissolve the solid material.

B) Wash Solution
Add the entire contents of the AIA-PACK WASH CONCENTRATE (100 mL) to approximately 2.0 L of CAP Class I water or the clinical laboratory reagent water (formerly NCCLS Type I) defined by CLSI C3-A4 guideline, mix well, and adjust the final volume to 2.5 L.

C) Diluent
Add the entire contents of the AIA-PACK DILUENT CONCENTRATE (100 mL) to approximately 4.0 L of CAP Class I water or the clinical laboratory reagent water (formerly NCCLS Type I) defined by CLSI C3-A4 guideline, mix well, and adjust the final volume to 5.0 L.

II. Calibration Procedure

A) Calibration Curve
The calibrators for use with the ST AIA-PACK FT4 are prepared gravimetrically and are compared to internal reference standards.
The calibration curve for ST AIA-PACK FT4 is stable for up to 90 days. Calibration stability is monitored by quality control performance and is dependent on proper reagent handling and TOSOH AIA System maintenance according to the manufacturer's instructions.
Recalibration may be necessary more frequently if controls are out of the established range for this assay or when certain service procedures are performed (e.g. temperature adjustment, sampling mechanism changes, maintenance of the wash probe, or detector lamp adjustment or change). For further information regarding instrument operation, consult the TOSOH AIA System Operator's Manual.
A sample calibration curve from AIA-1800 follows and shows the algorithm used for calculating results.



Appendix (IX)

1007471001-072E
Rev. 07/12

Attention

For North and South American Customers: Please refer to the AIA-AAM Docs on CD for the appropriate information.

Para los Clientes en Norte y Sur América: favor de referirse a los documentos AIA-AAM en Disco para la información apropiada.

Aos clientes da América do Norte e América do Sul: favor consultar os documentos do AIA-AAM que estão em CD para informações adequadas.

Pour les clients en Amérique du Nord et en Amérique du Sud: veuillez consulter les documents AIA-AAM sur le CD pour l' information appropriée.

ST AIA-PACK TT3

For Quantitative Measurement of total triiodothyronine (TT_3) in Serum or Heparinized Plasma

NAME AND INTENDED USE

ST AIA-PACK TT3 is designed for IN VITRO DIAGNOSTIC USE ONLY for the quantitative measurement of total triiodothyronine (TT_3) in human serum or heparinized plasma on TOSOH AIA System Analyzers.

SUMMARY AND EXPLANATION OF TEST

Triiodothyronine (T_3) and thyroid hormone (thyroxine; T_4) regulate a variety of biochemical processes throughout the body *(1)*. The majority of T_3 in circulation is produced enzymatically by monodeiodination of T_4 in the peripheral tissues, rather than from direct secretion from the thyroid gland *(2)*. Approximately one-third of all T_4 secreted is deiodinated to yield T_3 *(3)*.
Serum T_3 measurement can be a valuable component of a thyroid-function screening panel in diagnosing certain disorders of thyroid function in addition to conditions caused by iodide deficiency. Assays for T_3 are valuable in early detection of hyperthyroidism and for monitoring the efficacy of treatment for thyroid disorders *(4)*. A normal T_3 value in the presence of an elevated T_4 and/or free T_4 (FT_4) level may also help to rule out hyperthyroidism *(5)*.

PRINCIPLE OF THE ASSAY

The ST AIA-PACK TT3 is a competitive enzyme immunoassay which is performed entirely in the ST AIA-PACK TT3 test cups. Triiodothyronine, which is displaced from its binding proteins by ANS (8-anilino-1-naphthalene sulfonic acid), and free T_3 present in the test sample compete with enzyme-labeled T_3 for a limited number of binding sites on a T_3 specific antibody immobilized on magnetic beads. The beads are washed to remove the unbound enzyme-labeled T_3 and are then incubated with a fluorogenic substrate, 4-methylumbelliferyl phosphate (4-MUP). The amount of enzyme-labeled T_3 that binds to the beads is inversely proportional to the T_3 concentration in the test sample. A standard curve using a range of known standard concentrations is prepared and unknown T_3 concentrations are calculated using this curve.

MATERIAL PROVIDED (ST AIA-PACK TT3, Cat. No. 0025282)

5 trays x 20 test cups
Plastic test cups containing lyophilized twelve magnetic beads with anti-T_3 sheep monoclonal antibody, 125 μL of T_3 conjugated to bovine alkaline phosphatase and ANS (8-anilino-1-naphthalene sulfonic acid) with sodium azide as a preservative.

MATERIALS REQUIRED BUT NOT PROVIDED

The following materials are required to perform triiodothyronine analysis using the ST AIA-PACK TT3 (Cat. No. 0025282) on the TOSOH AIA System Analyzers. They are available separately from TOSOH.

Materials			Cat. No.
AIA Nex-IA or AIA-21			0018539
AIA Nex-IA or AIA-21 LA			0018540
AIA-1800 ST			0019836
AIA-1800 LA			0019837
AIA-2000 ST			0022100
AIA-2000 LA			0022101
AIA-600 II			0019014
AIA-600 II BCR			0019328
AIA-900			0022930
AIA-360			0019945
AIA-PACK SUBSTRATE SET II			0020968
AIA-PACK SUBSTRATE REAGENT II			
AIA-PACK SUBSTRATE RECONSTITUENT II			
AIA-PACK TT3 CALIBRATOR SET			0020382
AIA-PACK TT3 CALIBRATOR (1)	0	ng/mL	
AIA-PACK TT3 CALIBRATOR (2)	0.5	ng/mL (approx.)	
AIA-PACK TT3 CALIBRATOR (3)	1.0	ng/mL (approx.)	
AIA-PACK TT3 CALIBRATOR (4)	2.0	ng/mL (approx.)	
AIA-PACK TT3 CALIBRATOR (5)	4.5	ng/mL (approx.)	
AIA-PACK TT3 CALIBRATOR (6)	9.0	ng/mL (approx.)	
AIA-PACK TT3 SAMPLE DILUTING SOLUTION			0020582
AIA-PACK WASH CONCENTRATE			0020955
AIA-PACK DILUENT CONCENTRATE			0020956
SAMPLE CUPS			0018581
AIA-PACK DETECTOR STANDARDIZATION TEST CUP			0020970
AIA-PACK SAMPLE TREATMENT CUP			0020971
Additional Requirements for AIA Nex-IA / AIA-21 only:			
PIPETTE TIPS			0018552
PRELOADED PIPETTE TIPS			0018583
Additional Requirements for AIA-600 II, AIA-900, AIA-1800 and AIA-2000:			
PIPETTE TIPS			0019215
TIP RACK			0019216
PRELOADED PIPETTE TIPS			0022103

Only materials obtained from TOSOH should be used. Materials obtained elsewhere should not be substituted since assay performance is characterized based strictly on TOSOH materials.

WARNINGS AND PRECAUTIONS

1. The ST AIA-PACK TT3 is intended for in vitro diagnostic use only.
2. Inspect the packaging and the exterior of the aluminum pouch for any sign of damage before use. If any damages are visible, contact your local TOSOH sales representative.
3. Test cups from different lots or different assays shall not be mixed within a tray.
4. The ST AIA-PACK TT3 contains sodium azide, which may react with lead or copper plumbing to form potentially explosive metal azides. When disposing of such reagents, always flush with large volumes of water to prevent azide build-up.
5. Human serum is not used in the preparation of this product; however, since human specimens will be used for samples and other quality control products in the lab may be derived from human serum, please use standard laboratory safety procedures in handling all specimens and controls.
6. Do not use beyond the expiration date.
7. For safe waste disposal, it is recommended that each laboratory complies with established laboratory procedures and local, state, and federal regulations.
8. After opening, the vial of AIA-PACK TT3 SAMPLE DILUTING SOLUTION should be kept tightly sealed with a clean rubber cap. Sealing with dirty material may cause deterioration of the reagent.
9. The remaining sample diluting solution after use should not be mixed with another vial but be discarded to avoid contamination.
10. Serum, dust, metal, or microorganism contamination may cause degradation of reconstituted substrate solution. Store in a clean environment, away from direct sunlight and ultraviolet light.
11. TOSOH recommends that a new pouch of the test cups should be used for calibration.

STORAGE AND STABILITY

All unopened materials are stable until the expiration date on the label when stored at the specified temperature.

Materials	Cat. No.
2-8°C:	
ST AIA-PACK TT3	0025282
AIA-PACK TT3 CALIBRATOR SET	0020382
AIA-PACK TT3 SAMPLE DILUTING SOLUTION	0020582
AIA-PACK SUBSTRATE SET II	0020968
AIA-PACK WASH CONCENTRATE	0020955
AIA-PACK DILUENT CONCENTRATE	0020956
1-30°C:	
AIA-PACK DETECTOR STANDARDIZATION TEST CUP	0020970
AIA-PACK SAMPLE TREATMENT CUP	0020971

After opening the aluminum pouch, ST AIA-PACK TT3 test cups can be left on-board of the TOSOH AIA System Analyzers (18-25°C) for a maximum of 10 days (10 x 24 hours). When stored over night at 2-8°C, the test cups can be used for up to 30 days (30 cycles of 8 hours on board and 16 hours in the refrigerator). Once the aluminum pouch is opened, the test cups must be used within 30 days.
AIA-PACK TT3 CALIBRATOR SET must be kept tightly sealed and refrigerated at 2-8°C. After opening, the calibrators should be used within 1 day.
After opening, AIA-PACK TT3 SAMPLE DILUTING SOLUTION can be left on-board of the TOSOH AIA System Analyzers (18-25°C) for a maximum of 2 days (2 x 24 hours). When stored over night at 2-8°C, the sample diluting solution can be used for up to 6 days (6 cycles of 8 hours on board and 16 hours in the refrigerator). The sample diluting solution should not be used beyond 90 days after opening, even if it is sealed and stored in the refrigerator.
Reconstituted substrate solution is stable for 3 days at 18-25°C or 30 days at 2-8°C. Working diluent and wash solutions are stable for 30 days at 18-25°C.
Reagents should not be used if they appear cloudy or discolored.

SPECIMEN COLLECTION AND HANDLING

1. Serum or heparinized plasma is required for the assay. EDTA and citrated plasma SHOULD NOT BE USED.
2. When using serum, a venous blood sample is collected aseptically without additives. Store at 18-25°C until a clot has formed (usually 15-45 minutes), then centrifuge to obtain the serum specimen for assay.
3. When using heparinized plasma, a venous blood sample is collected aseptically with designated additive. Centrifuge and separate plasma from the packed cells as soon as possible.
4. Inadequate centrifugation or the presence of fibrin or particulate matter in the sample may cause an erroneous result.
5. Samples containing inhibitors of alkaline phosphatase may cause erroneous results.
6. Inspect all samples for air bubbles and foaming. Remove any air bubbles prior to assay.
7. Specimen types should not be used interchangeably during serial monitoring of an individual patient. Measured concentrations may vary slightly between sample types in certain patients.
8. Samples may be stored at 2-8°C for up to 24 hours prior to analysis. If the analysis cannot be done within 24 hours, the sample should be stored frozen at –20°C or below for up to 60 days.
9. Repeated freeze-thaw cycles should be avoided. Turbid serum samples or samples containing particulate matter should be centrifuged prior to testing. Prior to assay, bring frozen samples to 18-25°C slowly and mix gently.
10. The sample required for analysis is 25 μL.

PROCEDURE

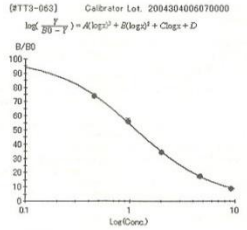
For the AIA Nex-IA / AIA-21, AIA-600 II, AIA-900, AIA-1800, AIA-2000 and AIA-360, please refer to their Operator's Manual for detailed instructions.

I. Reagent Preparation

A) Substrate Solution
Bring all reagents to 18-25°C before preparing the working reagent. Add the entire contents of the AIA-PACK SUBSTRATE RECONSTITUENT II (100 mL) to the lyophilized AIA-PACK SUBSTRATE REAGENT II and mix thoroughly to dissolve the solid material.
B) Wash Solution
Add the entire contents of the AIA-PACK WASH CONCENTRATE (100 mL) to approximately 2.0 L of CAP Class I water or the clinical laboratory reagent water (formerly NCCLS Type I) defined by CLSI C3-A4 guideline, mix well, and adjust the final volume to 2.5 L.
C) Diluent
Add the entire contents of the AIA-PACK DILUENT CONCENTRATE (100 mL) to approximately 4.0 L of CAP Class I water or the clinical laboratory reagent water (formerly NCCLS Type I) defined by CLSI C3-A4 guideline, mix well, and adjust the final volume to 5.0 L.

II. Calibration Procedure

A) Calibration Curve
The calibrators for use with the ST AIA-PACK TT3 are prepared gravimetrically and are compared to internal reference standards.
The calibration curve for ST AIA-PACK TT3 is stable for up to 90 days. Calibration stability is monitored by quality control performance and is dependent on proper reagent handling and TOSOH AIA System maintenance according to the manufacturer's instructions.
Recalibration may be necessary more frequently if controls are out of the established range for this assay or when certain service procedures are performed (e.g. temperature adjustment, sampling mechanism changes, maintenance of the wash probe, or detector lamp adjustment or change). For further information regarding instrument operation, consult the TOSOH AIA System Operator's Manual.
A sample calibration curve from AIA-1800 follows and shows the algorithm used for calculating results.



Appendix (X)

1003271001-033A
Rev. 03/13

ST AIA-PACK iFT3

For Quantitative Measurement of free triiodothyronine (FT_3) in Serum or Heparinized Plasma

NAME AND INTENDED USE

ST AIA-PACK iFT3 is designed for IN VITRO DIAGNOSTIC USE ONLY for the quantitative measurement of free triiodothyronine (FT_3) in human serum or heparinized plasma on TOSOH AIA System Analyzers.

SUMMARY AND EXPLANATION OF TEST

Triiodothyronine (T_3) is present in human serum in an equilibrium mixture of bound and free forms, with approximately 0.4% of the total T_3 circulating as free triiodothyronine (FT_3). Any change in the serum concentration of binding proteins will cause a parallel rise in the concentration of total T_3 with the FT_3 remaining relatively unchanged *(1)*. Its physiological action is apparent only when free fractions of T_3 and T_4 are taken in by target cell membrane receptors *(2-4)*. Since protein bound T_3 is not available for cellular uptake and does not appear to exert any metabolic effect, the measurement of FT_3 may therefore represent a more valid clinical assessment of patient thyroid status *(5-10)*. Direct measurement of free T_3 enables thyroid function examination even in the presence of abnormal liver function, hormone fluctuation during pregnancy *(11)*, and variations in levels of serum binding proteins.

PRINCIPLE OF THE ASSAY

The ST AIA-PACK iFT3 is a competitive enzyme immunoassay which is performed entirely in the ST AIA-PACK iFT3 test cups. Free triiodothyronine (FT_3) present in the test sample competes with enzyme-labeled triiodothyronine (T_3) for a limited number of binding sites on a T_3-specific antibody immobilized on magnetic beads. The beads are washed to remove the unbound enzyme-labeled free triiodothyronine and are then incubated with a fluorogenic substrate, 4-methylumbelliferyl phosphate (4MUP). The amount of enzyme-labeled free triiodothyronine that binds to the beads is inversely proportional to the free triiodothyronine concentration in the test sample. A standard curve using a range of known standard concentrations is constructed and unknown free triiodothyronine concentrations are calculated using this curve.

MATERIAL PROVIDED (ST AIA-PACK iFT3, Cat. No. 0025231)

5 trays x 20 test cups

Plastic test cups containing lyophilized twelve magnetic beads with anti-T_3 rabbit monoclonal antibody and 50 µL of T_3 conjugated to bovine alkaline phosphatase with sodium azide as a preservative.

MATERIALS REQUIRED BUT NOT PROVIDED

The following materials are required to perform free triiodothyronine analysis using the ST AIA-PACK iFT3 (Cat. No. 0025231) on the TOSOH AIA System Analyzers. They are available separately from TOSOH.

Materials			Cat. No.
AIA Nex·IA or AIA-21			0018539
AIA Nex·IA or AIA-21 LA			0018540
AIA-1800 ST			0019836
AIA-1800 LA			0019837
AIA-2000 ST			0022100
AIA-2000 LA			0022101
AIA-600 II			0019014
AIA-600 II BCR			0019328
AIA-900			0022930
AIA-360			0019945
AIA-PACK SUBSTRATE SET II			0020968
AIA-PACK SUBSTRATE REAGENT II			
AIA-PACK SUBSTRATE RECONSTITUENT II			
ST AIA-PACK iFT3 CALIBRATOR SET			0025331
ST AIA-PACK iFT3 CALIBRATOR (1)	0	pg/mL	
ST AIA-PACK iFT3 CALIBRATOR (2)	1.5	pg/mL (approx.)	
ST AIA-PACK iFT3 CALIBRATOR (3)	3.0	pg/mL (approx.)	
ST AIA-PACK iFT3 CALIBRATOR (4)	6.0	pg/mL (approx.)	
ST AIA-PACK iFT3 CALIBRATOR (5)	12	pg/mL (approx.)	
ST AIA-PACK iFT3 CALIBRATOR (6)	29	pg/mL (approx.)	
AIA-PACK WASH CONCENTRATE			0020955
AIA-PACK DILUENT CONCENTRATE			0020956
SAMPLE CUPS			0018581
AIA-PACK DETECTOR STANDARDIZATION TEST CUP			0020970
Additional Requirements for AIA Nex·IA / AIA-21 only:			
PIPETTE TIPS			0018552
PRELOADED PIPETTE TIPS			0018583
Additional Requirements for AIA-600 II, AIA-900, AIA-1800 and AIA-2000:			
PIPETTE TIPS			0019215
TIP RACK			0019216
PRELOADED PIPETTE TIPS			0022103

Only materials obtained from TOSOH should be used. Materials obtained elsewhere should not be substituted since assay performance is characterized based strictly on TOSOH materials.

WARNINGS AND PRECAUTIONS

1. The ST AIA-PACK iFT3 is intended for in vitro diagnostic use only.
2. Inspect the packaging and the exterior of the aluminum pouch for any sign of damage before use. If any damages are visible, contact your local TOSOH sales representative.
3. Test cups from different lots or different assays shall not be mixed within a tray.
4. The ST AIA-PACK iFT3 contains sodium azide, which may react with lead or copper plumbing to form potentially explosive metal azides. When disposing of such reagents, always flush with large volumes of water to prevent azide build-up.
5. The material derived from human origin is not used in the preparation of this product; however, since human specimens will be used for samples and other quality control products in the lab may be derived from human origin, please use standard laboratory safety procedures in handling all specimens and controls.
6. Do not use beyond the expiration date.
7. For safe waste disposal, it is recommended that each laboratory complies with established laboratory procedures and local, state, and federal regulations.
8. Serum, dust, metal, or microorganism contamination may cause degradation of reconstituted substrate solution. Store in a clean environment, away from direct sunlight and ultraviolet light.
9. TOSOH recommends that a new pouch of the test cups should be used for calibration.

STORAGE AND STABILITY

All unopened materials are stable until the expiration date on the label when stored at the specified temperature.

Materials	Cat. No.
2-8°C:	
ST AIA-PACK iFT3	0025231
ST AIA-PACK iFT3 CALIBRATOR SET	0025331
AIA-PACK SUBSTRATE SET II	0020968
AIA-PACK WASH CONCENTRATE	0020955
AIA-PACK DILUENT CONCENTRATE	0020956
1-30°C:	
AIA-PACK DETECTOR STANDARDIZATION TEST CUP	0020970

After opening the aluminum pouch, ST AIA-PACK iFT3 test cups can be left on-board of the TOSOH AIA System Analyzers (18-25°C) for a maximum of 4 days (4 x 24 hours). When stored over night at 2-8°C, the test cups can be used for up to 12 days (12 cycles of 8 hours on board and 16 hours in the refrigerator). Once the aluminum pouch is opened, even if the test cups are stored in the refrigerator, they must be used within 30 days.

ST AIA-PACK iFT3 CALIBRATOR SET must be kept tightly sealed and refrigerated at 2-8°C. After opening or reconstituting, the calibrators should be used within 1 day.

Reconstituted substrate solution is stable for 3 days at 18-25°C or 30 days at 2-8°C. Working diluent and wash solutions are stable for 30 days at 18-25°C.

Reagents should not be used if they appear cloudy or discolored.

SPECIMEN COLLECTION AND HANDLING

1. Serum or heparinized plasma is required for the assay. EDTA plasma or citrated plasma SHOULD NOT BE USED.
2. When using serum, a venous blood sample is collected aseptically with or without additives. Store at 18-25°C until a clot has formed (usually 15-45 minutes), then centrifuge to obtain the serum specimen for assay.
3. When using heparinized plasma, a venous blood sample is collected aseptically with the designated additive. Centrifuge and separate plasma from the packed cells as soon as possible.
4. Inadequate centrifugation or the presence of fibrin or particulate matter in the sample may cause an erroneous result.
5. Samples containing inhibitors of alkaline phosphatase may cause erroneous results.
6. Inspect all samples for air bubbles and foaming. Remove any air bubbles prior to assay.
7. Specimen types should not be used interchangeably during serial monitoring of an individual patient. Measured concentrations may vary slightly between sample types in certain patients.
8. Samples may be stored at 2-8°C for up to 7 days prior to analysis. If the analysis cannot be done within 7 days, the sample should be stored frozen at -20°C or below for up to 60 days.
9. Repeated freeze-thaw cycles should be avoided. Turbid serum samples or samples containing particulate matter should be centrifuged prior to testing. Prior to the assay, slowly bring frozen samples to 18-25°C and mix gently.
10. The sample required for analysis is 50 µL.

PROCEDURE

For the AIA Nex·IA / AIA-21, AIA-600 II, AIA-900, AIA-1800, AIA-2000, and AIA-360, please refer to their Operator's Manual for detailed instructions.

I. Reagent Preparation

A) Substrate Solution

Bring all reagents to 18-25°C before preparing the working reagent. Add the entire contents of the AIA-PACK SUBSTRATE RECONSTITUENT II (100 mL) to the lyophilized AIA-PACK SUBSTRATE REAGENT II and mix thoroughly to dissolve the solid material.

B) Wash Solution

Add the entire contents of the AIA-PACK WASH CONCENTRATE (100 mL) to approximately 2.0 L of CAP Class I water or the clinical laboratory reagent water (formerly NCCLS Type I) defined by CLSI C3-A4 guideline, mix well, and adjust the final volume to 2.5 L.

C) Diluent

Add the entire contents of the AIA-PACK DILUENT CONCENTRATE (100 mL) to approximately 4.0 L of CAP Class I water or the clinical laboratory reagent water (formerly NCCLS Type I) defined by CLSI C3-A4 guideline, mix well, and adjust the final volume to 5.0 L.

II. Calibration Procedure

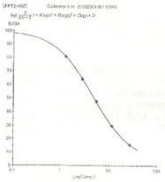
A) Calibration Curve

The calibrators for use with the ST AIA-PACK iFT3 are prepared gravimetrically and are compared to internal reference standards.

The calibration curve for ST AIA-PACK iFT3 is stable for up to 90 days. Calibration stability is monitored by quality control performance and is dependent on proper reagent handling and TOSOH AIA System maintenance according to the manufacturer's instructions.

Recalibration may be necessary more frequently if controls are out of the established range for this assay or when certain service procedures are performed (e.g. temperature adjustment, sampling mechanism changes, maintenance of the wash probe, or detector lamp adjustment or change). For further information regarding instrument operation, consult the TOSOH AIA System Operator's Manual.

A sample calibration curve from AIA-2000 follows and shows the algorithm used for calculating results.



B) Calibration Procedure

1. Refer to the appropriate TOSOH AIA System Operator's Manual for the procedural instructions.
2. Verify that both the calibrator lot and concentration numbers have been correctly entered into the software.
3. The ST AIA-PACK iFT3 CALIBRATOR SET is lyophilized. All levels should be reconstituted with 1.0 mL of CAP Class I water or the clinical laboratory reagent water (formerly NCCLS Type I) defined by CLSI C3-A4 guideline.
4. TOSOH recommends that all calibrators be run in triplicate.

C) Calibration Acceptability Criteria

1. Since there is an inverse relationship between concentration and rate, the rate should decrease as the concentration increases.
2. The replicate values should be within a 10% range.

D) Calibration Review and Acceptance

1. Review the calibration curve carefully, using the criteria listed above.
2. Edit the calibration if necessary, then accept the calibration.

For further information regarding calibration, consult the TOSOH AIA System Operator's Manual.

Appendix (XI)

fortress diagnostics
ISO 13485 accredited company

BXEO896A
96 TESTS
STORE AT 2-8°C
FOR IN-VITRO DIAGNOSTICS USE ONLY

Anti-Thyroid Peroxidase
(Anti-TPO)
ELISA

Intended Use:
For the quantitative determination of Thyroid Peroxidase (TPO) Autoantibodies in human serum of plasma by a Microplate Enzyme Immunoassay. Measurements of TPO Autoantibodies may aid in the diagnosis of certain thyroid diseases such as Hashimoto's and Grave's as well as nontoxic goitre.

Summary and explanation of the Test:
Antibodies to thyroid Peroxidase ha (95%), idiopathic myedema (90%) and Graves Disease (80%). In fact 72% of patients positive for anti-TPO exhibit some degree of thyroid dysfunction. This has lead to the clinical measurement becoming a valuable tool in the diagnosis of thyroid dysfunction.
Measurements of antibodies to TPO have been done, in the past, by Passive Haemaglutination (PHA). PHA tests do not have the sensitivity of enzyme immunoassay and are limited by subjective interpretation. This procedure, with the enhanced sensitivity of EIA, permits the delectability of subclinical levels of antibodies to TPO. In addition, the results are quantitated by a spectrophotometer, which eliminates subjective interpretation.
Fortress's microplate enzyme immunoassay methodology provides the technician with optimum sensitivity while requiring few technical manipulations. In the method, serum reference, diluted patient specimen, or control is first added to a microplate well. Biotinylated Thyroid Peroxidase Antigen (TPO) is added, and then the reactants are mixed. Reaction results between the antibodies to TPO and the biotinylated TPO to form an immune complex, which is deposited to the surface of streptavidin coated wells through the high affinity reaction of biotin and streptavidin. After the completion of the required incubation period, aspiration of decantation separates the reactants that are not attached to the wells. An enzyme anti-human IgG conjugate is then added to permit quantitation of reaction through interacting with human IgG of the immune complex. After washing, the enzyme activity is determined by reaction with substrate to produce colour. The employment of several serum references of known antibody activity permits construction of a graph of enzyme and antibody activities. From comparison to the dose response curve, an unknown specimens enzyme activity can be correlated with autoimmune antibody level.

Principle Of The Test:
A sequential Elisa Method (type 1):
The reagents required for the sequential ELISA assay include immobilizes antigen, circulating autoantibody and enzyme-linked species-specific antibody. In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated thyroid peroxidase antigen. Upon mixing biotinylated antibody, and a serum containing the autoantibody, a reaction results between the antigen and the antibody to form an immune-complex.
The interaction is illustrated by the following equation:

$$h\text{-}Ab_{(x\text{-}TPO)} + {}^{Btn}Ag_{(TPO)} \underset{K_{-a}}{\overset{K_a}{\rightleftharpoons}} h\text{-}Ab_{(x\text{-}TPO)} - {}^{Btn}Ag_{(TPO)}$$

$^{Btn}Ag_{(TPO)}$ = Biotinylated Antigen (Constant Quantity)
$h\text{-}Ab_{(x\text{-}TPO)}$ = Human Auto-Antibody (Variable Quantity)
$Ab_{(x\text{-}TPO)} - {}^{Btn}Ag_{(TPO)}$ = Immune Complex (Vairable Quantity)
K_a = Rate Constant of Association
K_{-a} = Rate Constant of Disassociation
Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody.
This interaction is illustrated below:

$$h\text{-}Ab_{(x\text{-}TPO)}\text{-}{}^{Btn}Ag_{(TPO)} + Streptavidin_{c.w} \Rightarrow \underline{Immobilized\ complex}\ (IC)$$

Streptavin c.w. = Streptavidin immobilized on well
Immobilized complex (IC) = sandwich complex bound to the solid surface.

After a suitable incubation period, the well is washed to separate the unbound components by aspiration and/or decantation. The enzyme linked species-specific antibody (anti-h-IgG) is then added to the microwells. This conjugates binds to the immune complex that formed.

$$I.C_{(h\text{-}IgG)} + {}^{ENZ}Ab_{(x\text{-}h\text{-}IgG)} + {}^{ENZ}Ab_{(x\text{-}h\text{-}IgG)}\text{-}I.C_{(h\text{-}IgG)}$$

$I.C._{(h\text{-}IgG)}$ = Immobilized immunce Complex (Variable Quantity)
$^{ENZ}Ab_{(x\text{-}h\text{-}IgG)}$ = Enzyme- antibody Conjugate (Constant Quantity)
$^{ENZ}Ab_{(x\text{-}h\text{-}IgG)}\text{-}I.C._{(h\text{-}IgG)}$ = Ag-Ab Complex (variable Quantity)

The anti-h-IgG enzyme conjugate that binds to the immune complex in a second incubation is separated from unreacted material by a wash step. The enzyme activity in this fraction is directly proportional to the antibody concentration in the specimen. By utilizing several different serum references of known antigen concentration, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

REAGENTS
Provided: Store at 2-8°C

(Anti –TPO) Kit Contents;	Volume
x-TPO Calibrators 6 levels as mentioned on the label.	6 x1ml
TPO Biotin Reagent.	1x13ml
Anti-TPO Enzyme Reagent.	1x13ml
Streptavidin coated Microplate.	96 Wells
Wash Solution Concentrate.	1x 20ml
Serum Diluent.	1x20ml
Substrate A	1x7ml
Substrate B	1x7ml
Stop Solution	1x8ml
Product Insert	1

Note 1: Do not use reagents beyond the kit expiration date.
Note 2: Opened reagents are stable for sixty (60) days. when stored at 2-8°C.
Note 3: Above reagents are for a single 96-well microplate.
Note 4: Calibrators are human serum based, were calibrated using 1st International ref. preparation which was assayed against MRC research standard A65/93 for anti-thyroid Peroxidase activity.

Required But Not Provided:
1. Pipette capable of delivering 10, 25 & 50µl volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100ml and 0.300ml volumes with a precision of better than 1.5%.
3. Microplate washers or a squeeze bottle (optional).
4. Microplate Reader with 450nm and 620nm wavelength absorbance capability.
5. Absorbent Paper for blotting the microplate wells.
6. Plastic wrap or microplate cover for incubation steps.
7. Vacuum aspirator (optional) for wash steps.
8. Test tube(s) of patient dilution
9. Timer.
10. Quality control materials.

PRECAUTIONS
For In Vitro Diagnostic Use
Not for Internal or External Use in Humans or Animals
All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies by FDA licensed reagents. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories,"2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.

SPECIMEN COLLECTION AND PREPARATION
The specimens shall be blood; serum in type and the usual precautions in the collection of venipuncture samples should be observed. For accurate comparison to established normal values, a fasting morn sample should be obtained. The blood s collected in a plain redtop venipuncture tut additives or anti-coagulants. Allow the blood samples.Centrifuge the specimen to separate from the cells. Samples may be refrigerated at maximum period of five (5) days. If the sp cannot be assayed within this time, the sample stored at temperatures of -20°C for up to 30 d repetitive freezing and thawing. When a duplicate, 0.050ml of the specimen is required.

REAGENT PREPARATION:
1. **Serum Diluent**
Dilute the serum diluents to 200ml in a suitable with distilled or deionized water. Store at (2-8°C
2. Wash buffer
Dilute contents of wash concentrate to 1(distilled or deionized water is a suitable storage Store at room temperature 20-27°C for up to 60
3. **Working Substrate Solution**
Pour the contents of the vial labeled Solution ' vial labeled Solution 'B'. Place the yellow c clear vial for easy identification. Mix c accordingly store at 2-8°C.
Note: Do not use the working substrate if it looks
4. Patient Sample Dilution (1/100)
Dispense 0.010ml (10µl) of each patient spec 1ml of serum diluent Cover and vortex or mix by inversion. Store at 2-8°C for up to forty-eight

TEST PROCEDURE
Before proceeding with the assay, bring all serum references and controls to room temper 27° C).
1. Format the microplates' wells for ea reference, control and patient specimen to b in duplicate. **Replace any unused microwell s into the aluminum bag, seal and store at 2-8°C.**
2. Pipette 0.025 ml (25µl) of the appropri reference, control or specimen into the assigne
3. Add 0.100 ml (100µl) of the *TPO Biotin reage* well.
4. Swirl the microplate gently for 20-30 seco and cover.
5. Incubate 60 minutes at room temperature.
6. Discard the contents of the microplate by de or aspiration. If decanting, tap and blot the pla absorbent paper.
7. Add 300µl of wash buffer (see Reagent Prepc Section), decant (tap and blot) or aspirate. R (2) additional times for a total of three (3) wash
An automatic or manual plate washer can Follow the manufacturer's instruction for proper squeeze bottle is employed, fill each well by the container (avoiding air bubbles) to dis wash. Decant the wash and repeat two (2) times.
8. Add 0.100 ml (100µl) of the TPO Enzyme Rec wells. Always add reagents in the same order t reaction time differences between wells.
DO NOT SHAKE THE PLATE AFTER ENZYME ADDITI(
9. Incubate 30 minutes at room temperature.
10. Repeat steps (6&7) as explained above

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fortress diagnostics
ISO 13485 accredited company

BXEO895A
96 TESTS
STORE AT 2-8°C
FOR IN-VITRO DIAGNOSTICS USE ONLY

Anti-Thyroglobulin
(Anti – Tg)
ELISA

Intended Use:
For the quantitative determination of Thyroglobulin (Tg) Autoantibodies in human serum of plasma by a Microplate Enzyme Immunoassay. Measurements of Tg Autoantibodies may aid in the diagnosis of certain thyroid diseases such as Hashimoto's and Grave's as well as nontoxic goitre.

Summary and explanation of the Test:

Antibodies to thyroglobulin have been shown to be characteristically present from patients with thyroiditis and primary thyrotoxicosis. This has led to the clinical measurement becoming a valuable tool in the diagnosis of thyroid dysfunction. Passive haemagglutination (PHA) methods have been employed in the past for measurements of antibodies to Tg. PHA tests to not have the sensitivity of EIA and are limited by subjective interpretation. This procedure with the enhanced sensitivity of EIA permits the detectability of subclinical levels of antibodies to Tg. In addition, the results are quantitated by a spectrophotometer, which eliminates subjective interpretation.

Fortress's microplate enzyme immunoassay methodology provides the technician with optimum sensitivity while requiring few technical manipulations. In the method, serum reference, diluted patient specimen, or control is first added to a microplate well. Biotinylated Thyroglobulin (Tg) is added, and then the reactants are mixed. Reaction results between the antibodies to Tg and the biotinylated Tg to form an immune complex, which is deposited to the surface of streptavidin coated wells through the high affinity reaction of biotin and streptavidin.

After the completion of the required incubation period, aspiration of decantation separates the reactants that are not attached to the wells. An enzyme anti-human IgG conjugate is then added to permit quantitation of reaction through interacting with human IgG of the immune complex. After washing, the enzyme activity is determined by reaction with substrate to produce colour. The employment of several serum references of known antibody activity permits construction of a graph of enzyme and antibody activites. From comparison to the dose response curve, an unknown specimens enzyme activity can be correlated with autoimmune antibody level.

Principle of The Test:
A sequential Elisa Method (type 1):
The reagents required for the sequential ELISA assay include immobilizes antigen, circulating autoantibody and enzyme-linked species-specific antibody. In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated thyroid peroxidase antigen.
Upon mixing, biotinylated antibody, and a serum containing the autoantibody, a reaction results between the antigen and the antibody to form an immune-complex.
The interaction is illustrated by the following equation:

$$h\text{-}Ab_{(x\text{-}Tg)} + {}^{Btn}Ag_{(Tg)} \underset{K_{-a}}{\overset{K_a}{\rightleftharpoons}} h\text{-}ab_{(x\text{-}Tg)} - {}^{Btn}Ag_{(Tg)}$$

$^{Btn}Ag_{(Tg)}$ = Biotinylated Antigen (Constant Quantity)
$h\text{-}Ab_{(x\text{-}Tg)}$ = Human Auto-Antibody (Variable Quantity)
$Ab_{(x\text{-}Tg)}\text{-}{}^{Btn}Ag_{(Tg)}$ =Immune Complex(Variable Quantity)
K_a = Rate Constant of Association
K_{-a} = Rate Constant of Disassociation

Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody.
This interaction is illustrated below:
h-Ab(x-Tg) – Btn Ag(Tg)+Streptavidin C.W ⇒ immobilized complex (IC) Streptavidin C.W = Streptavidin immobilized on well
Immobilized complex (IC) = Sandwich complex bound to the solid surface.
After a suitable incubation period, the well is washed to separate the unbound components by aspiration and/or decantation. The enzyme linked species-specific antibody (anti-h-IgG) is then added to the microwells. This conjugates binds to the immune complex that formed.

I.C.(h-IgG) + ENZ Ab (x-h-IgG) ⇒ ENZ Ab (⁎h-IgG)-I.C (h-IgG)
I.C. (h-IgG) = Immobilized Immune complex (Variable quantity)
ENZ Ab 9x-h-IgG) = Enzyme – antibody Conjugate (Constant Quantity)
ENZ Ab (x-h-IgG) – I.C. (h-IgG) = Ag – Ab Complex (Variable Quantity)

The anti-h-IgG enzyme conjugate that binds to the immune complex in a second incubation is separated from unreacted material by a wash step. The enzyme activity in this fraction is directly proportional to the antibody concentration in the specimen. By utilizing several different serum references of known antigen concentration, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

REAGENTS
Provided: Store at 2-8°C

Anti -Tg Kit Contents:	Volume
Anti-Tg Calibrators 6 levels as mentioned in the label.	6 x1ml
Thyroglobuline Biotin Reagent.	1x13ml
X-Tg Enzyme Reagent.	1x13ml
Streptavidin coated Microplate	96 Wells
Wash Solution Concentrate.	1x 20ml
Serum Diluent.	1x20ml
Substrate A	1x7ml
Substrate B	1x7ml
Stop Solution.	1x8ml
Product Insert	1

Note 1: Do not use reagents beyond the kit expiration date.
Note 2: Opened reagents are stable for sixty (60) days when stored at 2-8°C.
Note 3: Above reagents are for a single 96-well microplate.
Note 4: Calibrators are human serum based, were calibrated using 1st International ref. preparation which was assayed against MRC research standard A65/93 for anti-thyroglobulin activity.

Required But Not Provided:
1. Pipette capable of delivering 10 & 50µl volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100ml and 0.300ml volumes with a precision of better than 1.5%.
3. Microplate washers or a squeeze bottle (optional).
4. Microplate Reader with 450nm and 620nm wavelength absorbance capability.
5. Absorbent Paper for blotting the microplate wells.
6. Plastic wrap or microplate cover for incubation steps.
7. Vacuum aspirator (optional) for wash steps.
8. Test tube(s) of patient dilution
9. Timer.
10. Quality control materials.

PRECAUTIONS
For In Vitro Diagnostic Use
Not for Internal or External Use in Humans or Animals
All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV
Antibodies by FDA licensed reagents. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Micro and Biomedical Laboratories," 2nd Edition, Publication No. (CDC) 88-8395.

SPECIMEN COLLECTION AND PREPARATION
The specimens shall be blood; serum in type usual precautions in the collection of ve samples should be observed. For accurate con established normal values, a fasting morn sample should be obtained. The blood s collected in a plain red top venipuncture tut additives or anti-coagulants. Allow the blood samples.
Centrifuge the specimen to separate the serur cells. Samples may be refrigerated at 2-8 maximum period of five (5) days. If the sp cannot be assayed within this time, the sample stored at temperatures of -20°C for
up to 30 days. Avoid repetitive freezing and When assayed in duplicate, 0.050ml of the sp required.

REAGENT PREPARATION:
1. Serum Diluent
Dilute the serum diluents to 200ml in a suitable with distilled or deionized water. Store at (2-8°C
2. Wash buffer
Dilute contents of wash concentrate to 1C distilled or deionized water is a suitable storage Store at room temperature 20-27°C for up to 60
3. Working Substrate Solution
Pour the contents of the vial labeled Solution ' vial labeled Solution 'B'. Place the yellow c clear vial for easy identification. Mix c accordingly store at 2-8°C.
Note: Do not use the working substrate if it looks
4. Patient Sample Dilution (1/100)
Dispense 0.010ml (10µl) of each patient spe 1ml of serum diluent Cover and vortex or mix by inversion. Store at 2-8°C for up to forty-eight

TEST PROCEDURE
Before proceeding with the assay, bring all serum references and controls to room temperc (20 - 27° C).
1. Format the microplates' wells for ea reference, control and patient specimen to b in duplicate. **Replace any unused microwell s into the aluminum bag, seal and store at 2-8°C.**
2. Pipette 0.050 ml (50µl) of the appropri reference, control or specimen into the assigne
3. Add 0.100 ml (100µl) of the *Tg Biotin reager* well.
4. Swirl the microplate gently for 20-30 seco and cover.
5. Incubate 60 minutes at room temperature.
6. Discard the contents of the microplate by de or aspiration. If decanting, tap and blot the pla absorbent paper.
7. Add 300µl of wash buffer (see Reagent Prepc Section), decant (tap and blot) or aspirate. R (2) additional times for a total of three (3) wash **An automatic or manual plate washer can Follow the manufacturer's instruction for proper squeeze bottle is employed, fill each well by**

Fortress Diagnostics Limited, Unit 2C Antrim Technology Park, Antrim, BT41 1QS (United Kingdom)
Tel: +44 (0) 2894 487676 | Fax: +44 (0) 2894 469933 | Website: www.fortressdiagnostics.com

Appendix (XIII)

بسم الله الرحمن الرحيم

جمهورية السودان The Republic of the Sudan

وزارة التعليم العالي والبحث العلمي

Ministry of Higher Education & Scientific Research

جامعة شندي Shendi University

كلية الدراسات العليا والبحث العلمي

Faculty of Graduate Studies & Scientific Research

2015/8/9م النمرة :ج ش/ ك د ع/أ/١

الأخ/المدير العام لمستشفى الملك نمر الجامعي

الموقر،،،

السلام عليكم ورحمه الله وبركاته

الموضوع: تسهيل إجراءات دراسة دكتوراه

إشارة للموضوع أعلاه نفيدكم بأن الطالب/**عبد الوهاب عابدين سعيد طه** من ضمن الطلاب المسجلين لنيل درجه الدكتوراه في علوم المختبرات الطبية (تخصص كيمياء سريرية).

ونأمل في حسن تعاونكم مع كلية الدراسات العليا والبحث العلمي جامعة شندي، نرجو شاكرين تسهيل مهمته بغرض إجراءات البحث بجمع عينات لتنفيذ الجانب العملي من رسالة الدكتوراه .

ولكم فائق شكرنا وتقديرنا،،،

أ. د. سيف الدين الياس حمدتو أرباب

عميد كلية الدراسات العليا والبحث العلمي

جامعة شندي - عميد كلية الدراسات العليا والبحث العلمي - كلية الدراسات العليا

السودان- شندي- ص.ب: ١٤٣/١٤٢ – هاتف: ٠٠٢٤٩١٥٥٦٦٢١٦٧ – فاكس: ٠٠٢٤٩٢٦١٨٧٢٥٠٩

Sudan – Shendi – B.O.Box:142-143 Tel: +249155662167 – Fax+249-261872509 – e-mail:fgs@ush.sd

Appendix (XIV)

بسم الله الرحمن الرحيم

جامعة شندي

مستشفي الملك نمر الجامعي

الموضوع/ موافقة المؤسسة الصحية علي إجراء البحث

بالإشارة للموضوع اعلاه و بعد الاطلاع علي البحث المقدم من الطالب / عبد الوهاب عابدين سعيد و ان اجراء البحث ليس له اي تاثيرات جانبية علي المرضي لذلك لا مانع لادارة المستشفي من اخذ العينات من المرضي شريطة اخذ موافقتهم بعد شرح تفاصيله الكاملة و فوائده.

اسم مدير المستشفي ..... التوقيع .....

التاريخ : ٥/ ٩/ ٢٠١٥م

جامعة شندي
التاريخ ٥/٩/٢٠١٥م
مستشفى الملك نمر الجامعي

Appendix (XV)

بسم الله الرحمن الرحيم

إقـــــــــرار بالمــوافقة

الإسم: ---------- ----------

العمر: ---------- العنوان: ----------

أوافق بمحض إرادتي بالمشاركة في البحث العلمي المتعلق بدراسة تقييم الهرمونات والاجسام المضادة الخاصة بالغدة الدرقية لدي المصابين بامراض الغدة الدرقية الغير سرطانية بمنطقة محلية شندي

إعداد الطالب: عبد الوهاب عابدين سعيد طه

بعد أن شرح لي بأنه لا يترتب عليه أي أذى جسدي أو نفسي وأعلم أن المشاركة في هذا البحث لن يؤثر بأي حال من الأحوال في الرعاية الطبية التي أتلقاها كما أنه يحق لي الإنسحاب بدون ابداء اسباب الانسحاب من هذا البحث في أي مرحله من مراحله

البحث بإشراف/ أ.د. راشد الطيب عبد الله

التوقيع: ---------- التاريخ: ----------

Appendix (XVI)

6.2.7 Sudan map

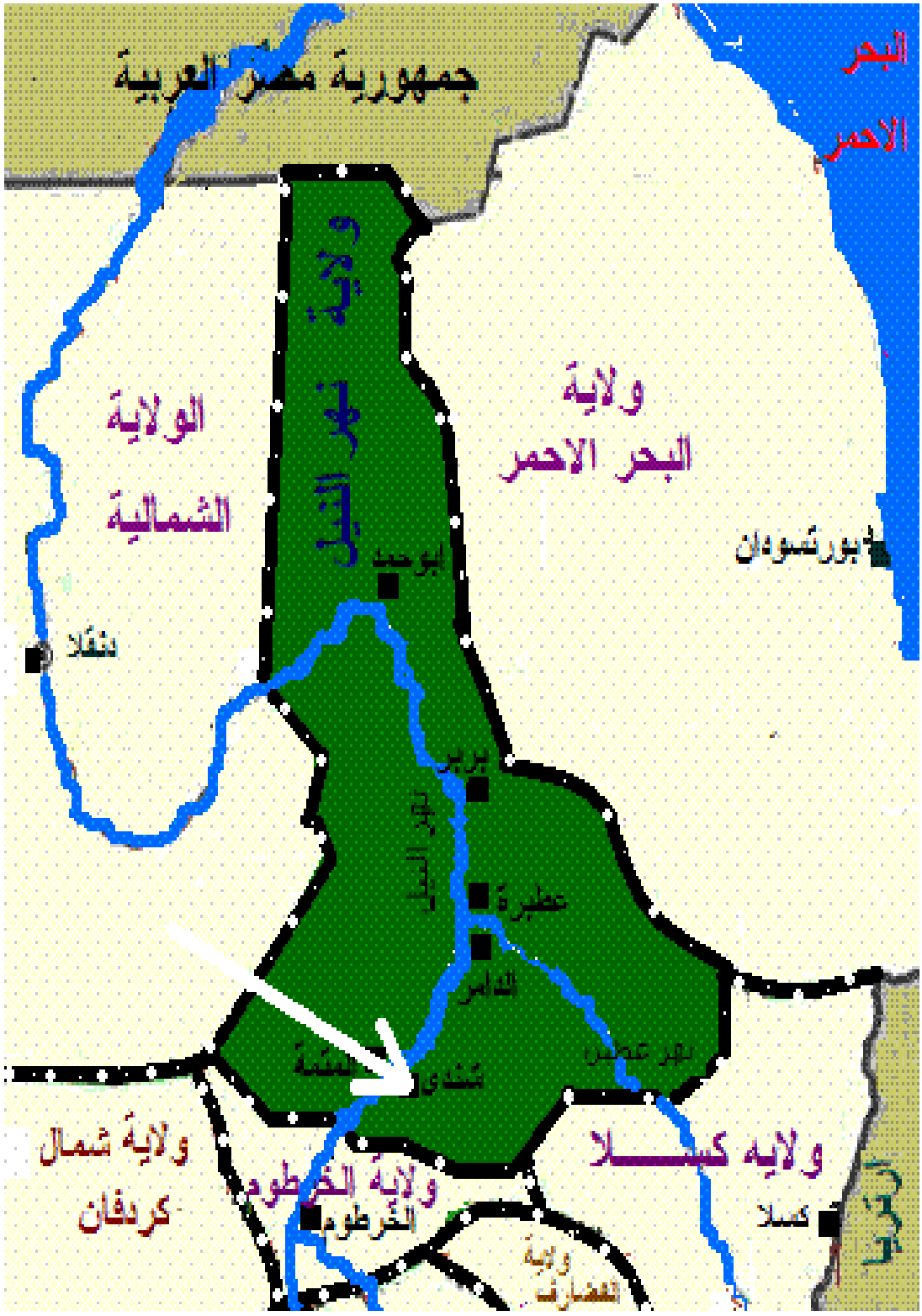

Table (4.59) Cumulative results

Clinical finding	N=111							N=72					
	Hypothyroidism							Hyperthyroidism					
	p.value							p.value					
	TSH	TT4	fT4	TT3	fT3	TPOAb	TgAb	TT4	fT4	TT3	fT3	TPOAb	TgAb
Restlessness					Significant								
Sweating												Significant	Significant
Tremor													Significant
Diarrhea						Significant					Significant		
Fatigue	Significant	Significant	Significant	Significant								Significant	
Weight loss													Significant
Increase appetites												Significant	
Heat intolerance						Significant	Significant						
Fever												Significant	Significant
Anorexia													Significant
Lid lags													Significant
Lid retraction													Significant
Exophthalmoplagia													Significant
Proximal myopathy				Significant	Significant								
Thick skin													Significant
Pretibial myexodema								Significant	Significant	Significant	Significant		

Clinical finding	N=111							N=72					
	Hypothyroidism							Hyperthyroidism					
	p.value							p.value					
	TSH	TT4	fT4	TT3	fT3	TPOAb	TgAb	TT4	fT4	TT3	fT3	TPOAb	TgAb
Change in voice	Significant			Significant	Significant		Significant						
Fine tremor												Significant	
Sweating of hands												Significant	Significant
Hotness											Significant		
Tachycardia										Significant			
Bradycardia	Significant												
Degree of F.H								Significant	Significant	Significant	Significant		
Sex	Significant	Significant	Significant	Significant	Significant								
Constipation			Significant		Significant								
Cold intolerance					Significant								
Slow relax reflex	Significant	Significant	Significant	Significant	Significant								